Exhibit 1
U.S. Patent Application Serial No. 08/976,560

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07H 17/00, C07K 14/00, A01N 43/04, A61K 35/14, C12Q 1/68, C12P 21/06, C12N 15/00 — A1

(11) International Publication Number: WO 99/47535

(43) International Publication Date: 23 September 1999 (23.09.99)

(21) International Application Number: PCT/US99/05606

(22) International Filing Date: 16 March 1999 (16.03.99)

(30) Priority Data:

60/078,044	16 March 1998 (16.03.98)	US
60/088,312	5 June 1998 (05.06.98)	US
60/106,056	28 October 1998 (28.10.98)	US
09/236,134	22 January 1999 (22.01.99)	US

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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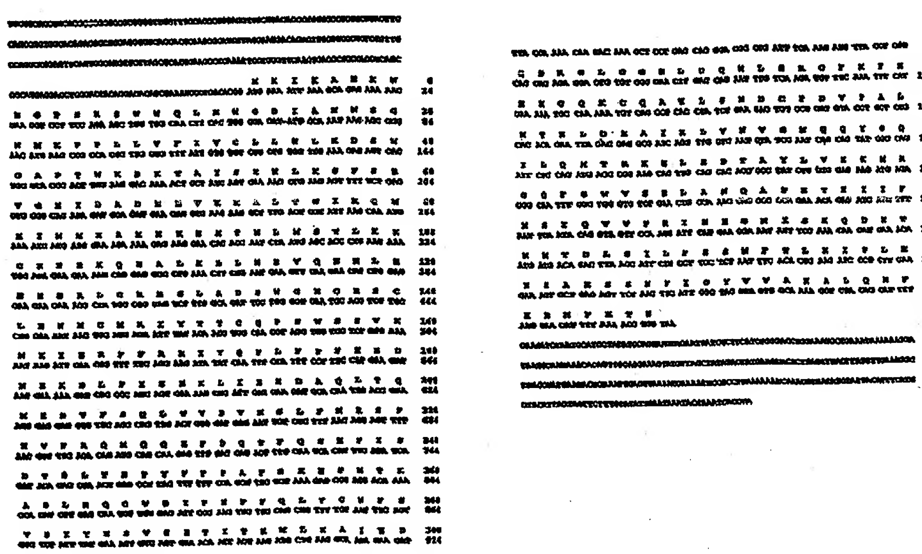
Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS AND COMPOSITIONS FOR DIAGNOSING AND TREATING CHROMOSOME-18p RELATED DISORDERS



(57) Abstract

The present invention relates to the mammalian *HKNG1* gene, a gene associated with bipolar affective disorder (BAD) in humans. The invention relates, in particular, to methods for the diagnostic evaluation, genetic testing and prognosis of HKNG1 neuropsychiatric disorders including schizophrenia, attention deficit disorder, a schizoaffective disorder, a bipolar affective disorder or a unipolar affective disorder.

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METHODS AND COMPOSITIONS FOR DIAGNOSING AND TREATING CHROMOSOME-18p RELATED DISORDERS

This is a continuation-in-part of U.S. application no. 09/236,134, filed on January 22, 1999 which claims priority under 35 U.S.C. § 119(e)(1) of provisional application no. 60/078,044 filed on March 16, 1998, of provisional application no. 60/088,312 filed on June 5, 1998, and of provisional application no. 60/106,056 filed on October 28, 1998, each of which is incorporated herein by reference in its entirety.

1. INTRODUCTION

The present invention relates, first, to the *HKNG1* gene, shown herein to be associated with central nervous system-related disorders, e.g., neuropsychiatric disorders, in particular, bipolar affective disorder and schizophrenia and with myopia-related disorders. The invention includes recombinant DNA molecules and cloning vectors comprising sequences of the *HKNG1* gene, and host cells and non-human host organisms engineered to contain such DNA molecules and cloning vectors. The present invention further relates to *HKNG1* gene products, and to antibodies directed against such *HKNG1* gene products. The present invention also relates to methods of using the *HKNG1* gene and gene product, including drug screening assays, and diagnostic and therapeutic methods for the treatment of *HKNG1*-mediated disorders, including *HKNG1*-mediated neuropsychiatric disorders such as bipolar affective disorder, as well as *HKNG1*-mediated myopia disorders such as early-onset autosomal dominant myopia.

2. BACKGROUND OF THE INVENTION

There are only a few psychiatric disorders in which clinical manifestations of the disorder can be correlated with demonstrable defects in the structure and/or function of the nervous system. Well-known examples of such disorders include Huntington's disease, which can be traced to a mutation in a single gene and in which neurons in the striatum degenerate, and Parkinson's disease, in which dopaminergic neurons in the nigro-striatal pathway degenerate. The vast majority of psychiatric disorders, however, presumably involve subtle and/or undetectable changes, at the cellular and/or molecular levels, in nervous system structure and function. This lack of detectable neurological defects distinguishes "neuropsychiatric" disorders, such as schizophrenia, attention deficit disorders, schizoaffective disorder, bipolar affective disorders, or unipolar affective disorder, from neurological disorders, in which anatomical or biochemical pathologies are manifest. Hence, identification of the causative defects and the neuropathologies of neuropsychiatric disorders are needed in order to enable clinicians to evaluate and prescribe appropriate courses of treatment to cure or ameliorate the symptoms of these disorders.

One of the most prevalent and potentially devastating of neuropsychiatric disorders is bipolar affective disorder (BAD), also known as bipolar mood disorder (BP) or manic-depressive illness, which is characterized by episodes of elevated mood (mania) and depression (Goodwin, *et al.*, 1990, *Manic Depressive Illness*, Oxford University Press, New York). The most severe and clinically distinctive forms of BAD are BP-I (severe bipolar affective (mood) disorder), which affects 2-3 million people in the United States, and SAD-M (schizoaffective disorder manic type). They are characterized by at least one full episode of mania, with or without episodes of major depression (defined by lowered mood, or depression, with associated disturbances in rhythmic

behaviors such as sleeping, eating, and sexual activity). BP-I often co-segregates in families with more etiologically heterogeneous syndromes, such as with a unipolar affective disorder such as unipolar major depressive disorder (MDD), which is a more broadly defined phenotype (Freimer and Reus, 1992, in *The Molecular and Genetic Basis of Neurological Disease*, Rosenberg, *et al.*, eds., Butterworths, New York, pp. 951-965; McInnes and Freimer, 1995, Curr. Opin. Genet. Develop., 5, 376-381). BP-I and SAD-M are severe mood disorders that are frequently difficult to distinguish from one another on a cross-sectional basis, follow similar clinical courses, and segregate together in family studies (Rosenthal, *et al.*, 1980, Arch. General Psychiat. 37, 804-810; Levinson and Levitt, 1987, Am. J. Psychiat. 144, 415-426; Goodwin, *et al.*, 1990, *Manic Depressive Illness*, Oxford University Press, New York). Hence, methods for distinguishing neuropsychiatric disorders such as these are needed in order to effectively diagnose and treat afflicted individuals.

Currently, individuals are typically evaluated for BAD using the criteria set forth in the most current version of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM). While many drugs have been used to treat individuals diagnosed with BAD, including lithium salts, carbamazepine and valproic acid, none of the currently available drugs are adequate. For example, drug treatments are effective in only approximately 60-70% of individuals diagnosed with BP-I. Moreover, it is currently impossible to predict which drug treatments will be effective in, for example, particular BP-I affected individuals. Commonly, upon diagnosis, affected individuals are prescribed one drug after another until one is found to be effective. Early prescription of an effective drug treatment, therefore, is critical for several reasons, including the avoidance of extremely dangerous manic episodes, the risk of progressive deterioration if effective

treatments are not found, and the risk of substantial side effects of current treatments.

The existence of a genetic component for BAD is strongly supported by segregation analyses and twin studies (Bertelson, *et al.*, 1977, Br. J. Psychiat. 130, 330-351; Freimer and Reus, 1992, in *The Molecular and Genetic Basis of Neurological Disease*, Rosenberg, *et al.*, eds., Butterworths, New York, pp. 951-965; Pauls, *et al.*, 1992, Arch. Gen. Psychiat. 49, 703-708). Efforts to identify the chromosomal location of genes that might be involved in BP-I, however, have yielded disappointing results in that reports of linkage between BP-I and markers on chromosomes X and 11 could not be independently replicated nor confirmed in the re-analyses of the original pedigrees, indicating that with BAD linkage studies, even extremely high lod scores at a single locus, can be false positives (Baron, *et al.*, 1987, Nature 326, 289-292; Egeland, *et al.*, 1987, Nature 325, 783-787; Kelsoe, *et al.*, 1989, Nature 342, 238-243; Baron, *et al.*, 1993, Nature Genet. 3, 49-55).

Recent investigations have suggested possible localization of BAD genes on chromosomes 18p and 21q, but in both cases the proposed candidate region is not well defined and no unequivocal support exists for either location (Berrettini, *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91, 5918-5921; Murray, *et al.*, 1994, Science 265, 2049-2054; Pauls, *et al.*, 1995, Am. J. Hum. Genet. 57, 636-643; Maier, *et al.*, 1995, Psych. Res. 59, 7-15; Straub, *et al.*, 1994, Nature Genet. 8, 291-296).

Mapping genes for common diseases believed to be caused by multiple genes, such as BAD, may be complicated by the typically imprecise definition of phenotypes, by etiologic heterogeneity, and by uncertainty about the mode of genetic transmission of the disease trait. With neuropsychiatric disorders there is even greater ambiguity in distinguishing

individuals who likely carry an affected genotype from those who are genetically unaffected. For example, one can define an affected phenotype for BAD by including one or more of the broad grouping of diagnostic classifications that constitute the mood disorders: BP-I, SAD-M, MDD, and bipolar affective (mood) disorder with hypomania and major depression (BP-II).

Thus, one of the greatest difficulties facing psychiatric geneticists is uncertainty regarding the validity of phenotype designations, since clinical diagnoses are based solely on clinical observation and subjective reports. Also, with complex traits such as neuropsychiatric disorders, it is difficult to genetically map the trait-causing genes because: (1) neuropsychiatric disorder phenotypes do not exhibit classic Mendelian recessive or dominant inheritance patterns attributable to a single genetic locus, (2) there may be incomplete penetrance, *i.e.*, individuals who inherit a predisposing allele may not manifest disease; (3) a phenocopy phenomenon may occur, *i.e.*, individuals who do not inherit a predisposing allele may nevertheless develop disease due to environmental or random causes; (4) genetic heterogeneity may exist, in which case mutations in any one of several genes may result in identical phenotypes.

Despite these difficulties, however, identification of the chromosomal location, sequence and function of genes and gene products responsible for causing neuropsychiatric disorders such as bipolar affective disorders is of great importance for genetic counseling, diagnosis and treatment of individuals in affected families.

3. SUMMARY OF THE INVENTION

The present invention relates, first, to the discovery, identification, and characterization of novel nucleic acid molecules that are associated with central nervous system-related disorders and processes, *e.g.*, human neuropsychiatric disorders, such as schizophrenia, attention deficit disorder, schizoaffective disorder, dysthymic disorder, major

depressive disorder, and bipolar affective disorder (BAD) including severe bipolar affective (mood) disorder (BP-I), bipolar affective (mood) disorder with hypomania and major depression (BP-II). The invention further relates to the discovery, identification, and characterization of proteins encoded by such nucleic acid molecules, or by degenerate, *e.g.*, allelic or homologous, variants thereof. The invention further relates to the discovery, identification, and characterization of novel nucleic acid molecules that are associated with human myopia or nearsightedness, such as early-onset, autosomal dominant myopia, as well as to the discovery, identification, and characterization of proteins encoded by such nucleic acid molecules or by degenerate variants thereof.

In particular, the nucleic acid molecules of the present invention represent, first, nucleic acid sequences corresponding to the gene referred to herein as *HKNG1*. As demonstrated in the Examples presented below in Sections 6, 8 and 14, the *HKNG1* gene is associated with human CNS-related disorders, *e.g.*, neuropsychiatric disorders, in particular BAD. The *HKNG1* gene is associated with other human neuropsychiatric disorders as well, such as schizophrenia. As demonstrated in the Example presented below in Section 14, the *HKNG1* gene is also associated with human myopia, such as early-onset autosomal dominant myopia.

In addition to the positive correlation between mutations within the *HKNG1* gene and individuals exhibiting symptoms of BAD, described in Section 6 and 8, the present invention is further based, in part, on Applicants' discovery of novel *HKNG1* cDNA sequences. Applicants' discovery of such cDNA sequences has led to the elucidation of the *HKNG1* genomic (that is, upstream untranslated, intron/exon, and downstream untranslated) structure, and to the discovery of full-length and alternately spliced *HKNG1* variants and the polypeptides encoded by such variants. These discoveries are

described in Sections 7 and 10, below. Applicants' discovery of such cDNA sequences has also led to the elucidation of novel mammalian (*e.g.*, guinea pig and bovine) *HKNG1* sequences, and to the discovery of novel allelic variants and polymorphisms of such sequences. These discoveries are described in Section 10 below.

The invention encompasses nucleic acid molecules which comprise the following nucleotide sequences: (a) nucleotide sequences (*e.g.*, SEQ ID NOS: 1, 3, 5, 6, 36, and 37) that comprise a human *HKNG1* gene and/or encode a human *HKNG1* gene product (*e.g.*, SEQ ID NO: 2; SEQ ID NO: 4), as well as allelic variants, homologs and orthologs thereof, including nucleotide sequences (*e.g.*, SEQ ID NOS:38, 40, 42, 44, and 46-48) that encode non-human *HKNG1* gene products (*e.g.*, SEQ ID NOS:39, 41, 43, 45, and 49); (b) nucleotide sequences comprising the novel *HKNG1* sequences disclosed herein that encode mutants of the *HKNG1* gene product in which sequences encoding all or a part of one or more of the *HKNG1* domains is deleted or altered, or fragments thereof; (c) nucleotide sequences that encode fusion proteins comprising a *HKNG1* gene product (*e.g.*, SEQ ID NO: 2; SEQ ID NO: 4), or a portion thereof fused to a heterologous polypeptide; and (d) nucleotide sequences within the *HKNG1* gene, as well as chromosome 18p nucleotide sequences flanking the *HKNG1* gene, which can be utilized, *e.g.*, as primers, in the methods of the invention for identifying and diagnosing individuals at risk for or exhibiting an *HKNG1*-mediated disorder, such as BAD or schizophrenia, or for diagnosing individuals at risk for or exhibiting a form of myopia such as early-onset autosomal dominant myopia. The nucleic acid molecules of (a) through (d), above, can include, but are not limited to, cDNA, genomic DNA, and RNA sequences.

The invention also encompasses the expression products of the nucleic acid molecules listed above; *i.e.*, peptides, proteins, glycoproteins and/or polypeptides that are encoded by the above *HKNG1* nucleic acid molecules.

The compositions of the present invention further encompass agonists and antagonists of the *HKNG1* gene product, including small molecules (such as small organic molecules), and macromolecules (including antibodies), as well as nucleotide sequences that can be used to inhibit *HKNG1* gene expression (*e.g.*, antisense and ribozyme molecules, and gene or regulatory sequence replacement constructs) or to enhance *HKNG1* gene expression (*e.g.*, expression constructs that place the *HKNG1* gene under the control of a strong promoter system).

The compositions of the present invention further include cloning vectors and expression vectors containing the nucleic acid molecules of the invention, as well as hosts which have been transformed with such nucleic acid molecules, including cells genetically engineered to contain the nucleic acid molecules of the invention, and/or cells genetically engineered to express the nucleic acid molecules of the invention. In addition to host cells and cell lines, hosts also include transgenic non-human animals (or progeny thereof), particularly non-human mammals, that have been engineered to express an *HKNG1* transgene, or "knock-outs" that have been engineered to not express *HKNG1*.

Transgenic non-human animals of the invention include animals engineered to express an *HKNG1* transgene at higher or lower levels than normal, wild-type animals. The transgenic animals of the invention also include animals engineered to express a mutant variant or polymorphism of an *HKNG1* transgene which is associated with *HKNG1*-mediated disorder, for example an *HKNG1*-mediated neuropsychiatric disorders, such as BAD and schizophrenia, or, alternatively, a myopia

disorder such as early-onset autosomal dominant myopia. The transgenic animals of the invention further include the progeny of such genetically engineered animals.

The invention further relates to methods for the treatment of *HKNG1*-mediated disorders in a subject, such as *HKNG1*-mediated neuropsychiatric disorders and *HKNG1*-mediated myopia disorders, wherein such methods comprise administering a compound which modulates the expression of a *HKNG1* gene and/or the synthesis or activity of a *HKNG1* gene product so symptoms of the disorder are ameliorated.

The invention further relates to methods for the treatment of *HKNG1*-mediated disorders in a subject, such as *HKNG1*-mediated neuropsychiatric disorders and *HKNG1*-mediated myopia disorders, resulting from *HKNG1* gene mutations or aberrant levels of *HKNG1* expression or activity, wherein such methods comprise supplying the subject with a nucleic acid molecule encoding an unimpaired *HKNG1* gene product such that an unimpaired *HKNG1* gene product is expressed and symptoms of the disorder are ameliorated.

The invention further relates to methods for the treatment of *HKNG1*-mediated disorders in a subject, such as *HKNG1*-mediated neuropsychiatric disorders and *HKNG1*-mediated myopia disorders, resulting from *HKNG1* gene mutations or from aberrant levels of expression or activity, wherein such methods comprise supplying the subject with a cell comprising a nucleic acid molecule that encodes an unimpaired *HKNG1* gene product such that the cell expresses the unimpaired *HKNG1* gene product and symptoms of the disorder are ameliorated.

The invention also encompasses pharmaceutical formulations and methods for treating *HKNG1*-mediated disorders, including neuropsychiatric disorders, such as BAD and schizophrenia, and myopia disorders, such as early-onset autosomal dominant myopia, involving *HKNG1* gene.

In addition, the present invention is directed to methods that utilize the *HKNG1* nucleic acid sequences, chromosome 18p nucleotide sequences flanking the *HKNG1* human gene and/or *HKNG1* gene product sequences for mapping the chromosome 18p region, and for the diagnostic evaluation, genetic testing and prognosis of a *HKNG1*-mediated disorder, such as a *HKNG1*-mediated neuropsychiatric disorder or a *HKNG1*-mediated myopia disorder. For example, in one embodiment, the invention relates to methods for diagnosing *HKNG1*-mediated disorders, wherein such methods comprise measuring *HKNG1* gene expression in a patient sample, or detecting a *HKNG1* polymorphism or mutation in the genome of a mammal, including a human, suspected of exhibiting such a disorder. In one embodiment, nucleic acid molecules encoding *HKNG1* can be used as diagnostic hybridization probes or as primers for diagnostic PCR analysis for the identification of *HKNG1* gene mutations, allelic variations and regulatory defects in the *HKNG1* gene which correlate with neuropsychiatric disorders such as BAD or schizophrenia.

The invention still further relates to methods for identifying compounds which modulate the expression of the *HKNG1* gene and/or the synthesis or activity of the *HKNG1* gene products, including therapeutic compounds, which reduce or eliminate the symptoms of *HKNG1*-mediated disorders, including *HKNG1*-mediated neuropsychiatric disorders such as BAD and schizophrenia. In particular, cellular and non-cellular assays are described that can be used to identify compounds that interact with the *HKNG1* gene product, e.g., modulate the activity of the *HKNG1* and/or bind to the *HKNG1* gene product. Such cell-based assays of the invention utilize cells, cell lines, or engineered cells or cell lines that express the *HKNG1* gene product.

In one embodiment, such methods comprise contacting a compound to a cell that expresses a *HKNG1* gene, measuring the level of *HKNG1* gene expression, gene product expression or gene product activity, and comparing this level to the level of *HKNG1* gene expression, gene product expression or gene product activity produced by the cell in the absence of the compound, such that if the level obtained in the presence of the compound differs from that obtained in its absence, a compound that modulates the expression of the *HKNG1* gene and/or the synthesis or activity of the *HKNG1* gene products has been identified.

In another embodiment, such methods comprise contacting a compound to a cell that expresses a *HKNG1* gene and also comprises a reporter construct whose transcription is dependent, at least in part, on *HKNG1* expression or activity. In such an embodiment, the level of reporter transcription is measured and compared to the level of reporter transcription in the cell in the absence of the compound. If the level of reporter transcription obtained in the presence of the compound differs from that obtained in its absence, a compound that modulates expression of *HKNG1* or genes involved in *HKNG1*-related pathways or signal transduction has been identified.

In yet another embodiment, such methods comprise administering a compound to a host, such as a transgenic animal, that expresses an *HKNG1* transgene or a mutant *HKNG1* transgene associated with an *HKNG1*-mediated disorder such as a neuropsychiatric disorder (*e.g.*, BAD or schizophrenia), or to an animal, *e.g.*, a knock-out animal, that does not express *HKNG1*, and measuring the level of *HKNG1* gene expression, gene product expression, gene product activity, or symptoms of an *HKNG1*-mediated disorder such as an *HKNG1*-mediated neuropsychiatric disorder (*e.g.*, BAD or schizophrenia). The

measured level is compared to the level obtained in a host that is not exposed to the compound, such that if the level obtained when the host is exposed to the compound differs from that obtained in a host not exposed to the compound, a compound modulates the expression of the mammalian *HKNG1* gene and/or the synthesis or activity of the mammalian *HKNG1* gene products, and/or the symptoms of an *HKNG1*-mediated disorder such as a neuropsychiatric disorder (*e.g.*, BAD or schizophrenia), has been identified.

The present invention still further relates to pharmacogenomic and pharmacogenetic methods for selecting an effective drug to administer to an individual having a *HKNG1*-mediated disorder. Such methods are based on the detection of genetic polymorphisms in the *HKNG1* gene or variations in *HKNG1* gene expression due to, *e.g.*, altered methylation, differential splicing, or post-translational modification of the *HKNG1* gene product which can affect the safety and efficacy of a therapeutic agent.

As briefly discussed above, the present invention is based, in part, on the genetic and physical mapping of the *HKNG1* gene to a specific portion of the short arm of human chromosome 18 that is associated with human neuropsychiatric disorders, in particular, bipolar affective disorder. These results are described in the Example presented, below, in Section 6. The invention is also based on the elucidation of the *HKGN1* nucleotide sequence, amino acid sequence and expression pattern, as described in the Example presented, below, in Section 7. The invention is further based on the identification of specific mutations and/or polymorphisms within the *HKNG1* gene which positively correlate with neuropsychiatric disorders, in particular, BAD, as described in the Example presented below in Section 8. These mutations include a point mutation discovered in an individual affected by BAD which is absent from the corresponding wild-type

nucleic acid derived from non-affected individuals and linkage disequilibrium of three markers showing cosegregation with a population of individuals with BAD. This mutation is a single base mutation which results in a mutant *HKNG1* gene product comprising substitution of a lysine residue for the wild-type glutamic acid residue at *HKNG1* amino acid position 202 of the polypeptide of SEQ ID NO:2 or the *HKNG1* amino acid residue 184 of the polypeptide of SEQ ID NO:4. These mutations further include the mutations discovered in schizophrenic and BAD patients that are detailed in FIGS. 5A-5B.

3.1. DEFINITIONS

As used herein, the following terms shall have the abbreviations indicated.

BAC, bacterial artificial chromosomes
BAD, bipolar affective disorder(s)
BP, bipolar mood disorder
BP-I, severe bipolar affective (mood) disorder
BP-II, bipolar affective (mood) disorder with hypomania and major depression
bp, base pair(s)
EST, expressed sequence tag
HKNG1, Hong Kong new gene 1
lod, logarithm of odds
MDD, unipolar major depressive disorder
ROS, reactive oxygen species
RT-PCR, reverse transcriptase PCR
SSCP, single-stranded conformational polymorphism
SAD-M, schizoaffective disorder manic type
STS, sequence tagged site
YAC, yeast artificial chromosome

"*HKNG1*-mediated disorders" include disorders involving an aberrant level of *HKNG1* gene expression, gene product

synthesis and/or gene product activity relative to levels found in clinically normal individuals, and/or relative to levels found in a population whose level represents a baseline, average HKNG1 level. While not wishing to be bound by any particular mechanism, it is to be understood that disorder symptoms can, for example, be caused, either directly or indirectly, by such aberrant levels. Alternatively, it is to be understood that such aberrant levels can, either directly or indirectly, ameliorate disorder symptoms, (*e.g.*, as in instances wherein aberrant levels of *HKNG1* suppress the disorder symptoms caused by mutations within a second gene).

HNKG1-mediated disorders include, for example, central nervous system (CNS) disorders. CNS disorders include, but are not limited to cognitive and neurodegenerative disorders such as Alzheimer's disease, senile dementia, Huntington's disease, amyotrophic lateral sclerosis, and Parkinson's disease, as well as Gilles de la Tourette's syndrome, autonomic function disorders such as hypertension and sleep disorders, and neuropsychiatric disorders that include, but are not limited to schizophrenia, schizoaffective disorder, attention deficit disorder, dysthymic disorder, major depressive disorder, mania, obsessive-compulsive disorder, psychoactive substance use disorders, anxiety, panic disorder, as well as bipolar affective disorder, *e.g.*, severe bipolar affective (mood) disorder (BP-I), bipolar affective (mood) disorder with hypomania and major depression (BP-II). Further CNS-related disorders include, for example, those listed in the American Psychiatric Association's Diagnostic and Statistical manual of Mental Disorders (DSM), the most current version of which is incorporated herein by reference in its entirety.

"*HKNG1*-mediated processes" include processes dependent and/or responsive, either directly or indirectly, to levels of *HKNG1* gene expression, gene product synthesis and/or gene

product activity. Such processes can include, but are not limited to, developmental, cognitive and autonomic neural and neurological processes, such as, for example, pain, appetite, long term memory and short term memory.

4. BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A-1B. The nucleotide sequence of human *HKNG1* cDNA (SEQ ID NO: 1) is depicted on the bottom line. The top line depicts the full length amino acid sequence of human *HKNG1* polypeptide (SEQ ID NO: 2) encoded by the human *HKNG1* cDNA sequence. The nucleotide sequence encoding SEQ ID NO:2 corresponds to SEQ ID NO:5.

FIG. 2A-2B. Nucleotide sequence of an alternately spliced human *HKNG1* variant, referred to as *HKNG1-V1* (SEQ ID NO: 3) is depicted on the bottom line. The derived amino acid sequence of the human *HKNG1* gene product (SEQ ID NO: 4) encoded by this alternately spliced cDNA variant is depicted on the top line. The nucleotide sequence encoding SEQ ID NO:4 corresponds to SEQ ID NO:6

FIG. 3A-3R. Genomic sequence of the human *HKNG1* gene (SEQ ID NO. 7). Exons are in bold and the 3' and 5' UTRs (untranslated regions) are underlined.

FIG. 4. Summary of in situ hybridization analysis of *HKNG1* mRNA distribution in normal human brain tissue.

FIGS. 5A-B. *HKNG1* polymorphisms relative to the *HKNG1* wild-type sequence. These polymorphisms were isolated from a collection of schizophrenic patients of mixed ethnicity from the United States (FIG. 5A) and from the San Francisco BAD collection (FIG. 5B).

FIGS. 6A-B. Nucleotide sequence of the RT-PCR products for *HKNG1*-V2 (FIG. 6A; SEQ ID NO:36) and *HKNG1*-V3 (FIG. 6B; SEQ ID NO:37).

FIG 7. The cDNA sequence (SEQ ID NO:38) and the predicted amino acid sequence (SEQ ID NO:39) of the guinea pig *HKNG1* ortholog *gphkng1815*.

FIG. 8. The cDNA sequence (SEQ ID NO:40) and the predicted amino acid sequence (SEQ ID NO:41) of *gphkng 7b*, an allelic variant of the guinea pig *HKNG1* ortholog *gphkng1815*.

FIG. 9. The cDNA sequence (SEQ ID NO:42) and the predicted amino acid sequence (SEQ ID NO:43) of *gphkng 7c*, an allelic variant of the guinea pig *HKNG1* ortholog *gphkng1815*.

FIG. 10. The cDNA sequence (SEQ ID NO:44)and the predicted amino acid sequence (SEQ ID NO:45) of *gphkng 7d*, an allelic variant of the guinea pig *HKNG1* ortholog *gphkng1815*.

FIG. 11. The cDNA sequence (SEQ ID NO:46) and the predicted amino acid sequence (SEQ ID NO:49) of the allelic variant *bhkng1* of the bovine *HKNG1* ortholog.

FIG. 12. The cDNA sequence (SEQ ID NO:47) and the predicted amino acid sequence (SEQ ID NO:49) of the allelic variant *bhkng2* of the bovine *HKNG1* homolgue.

FIG. 13. The cDNA sequence (SEQ ID NO:48) and the predicted amino acid sequence (SEQ ID NO:49) of the allelic variant *bhkng3* of the bovine *HKNG1* homolgue.

FIG. 14A-B. Alignments of the guinea pig *HKNG1* cDNA (FIG. 14A) and predicted amino acid (FIG. 14B) sequences for gphkng1815, gphkng 7b, gphkng7c, and gphkng 7d.

FIG. 15. Alignments of the cDNA sequences of the bovine *HKNG1* allelic variants *bhkng1*, *bhkng2*, and *bhkng3*.

FIG. 16. Alignments of the human (hkng_aa), bovine (bhkng1_aa) and guinea pig (gphkng1815_aa) HKNG1 amino acid sequences.

FIG. 17. Alignments of the human HKNG1 protein sequences; top line: the mature secreted HKNG1 protein sequence (SEQ ID NO:51); second line: immature HKNG1 protein form 1 (IPF1; SEQ ID NO:2); third line: immature HKNG1 protein form 2 (IPF2; SEQ ID NO:64); bottom line: immature HKNG1 protein form 3 (IPF3; SEQ ID NO:4).

FIG. 18. The nucleotide sequence of human *HKNG1* splice variant *HKNG1Δ7* cDNA (SEQ ID NO: 65) is depicted on the

bottom line. The top line depicts the full length amino acid sequence of human HKNG1Δ7 polypeptide (SEQ ID NO: 66) encoded by the human *HKNG1Δ7* cDNA sequence.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1. THE *HKNG1* GENE

HKNG1 nucleic acid molecules are described in the section. Unless otherwise stated , the term "HKNG1 nucleic acid" refers collectively to the sequences described herein.

A human *HKNG1* cDNA sequence (SEQ ID NO: 1) encoding the full length amino acid sequence (SEQ ID NO: 2) of the *HKNG1* polypeptide is shown in FIG. 1A-1B. The human *HKNG1* gene encodes a secreted polypeptide of 495 amino acid residues, as shown in FIG. 1A-1B, and SEQ ID NO: 2. The nucleotide sequence of the portion of the cDNA corresponding to the coding sequence for *HKNG1* (SEQ ID NO:2) is depicted as SEQ ID NO:5.

The *HKNG1* sequences of the invention also include splice variants of the *HKNG1* sequences described herein. For example, an alternately spliced human *HKNG1* cDNA sequence, referred to as *HKNG1-V1* (SEQ ID NO: 3) encoding a human *HKNG1* variant gene product (*i.e.*, the *HKNG1-V1* gene product) is shown in FIG. 2A-2B. This splice variant of a human *HKNG1* gene encodes a secreted polypeptide of 477 amino acid residues, as shown in FIG. 2A-2B, and SEQ ID NO:4. The nucleotide sequence of the portion of the cDNA corresponding to the coding sequence for *HKNG1* (SEQ ID NO:4) is depicted in SEQ ID NO:6.

Another alternately spliced human *HKNG1* cDNA sequence (SEQ ID NO:65), referred to as *HKNG1Δ7*, encodes a second *HKNG1* variant gene product (the *HKNG1Δ7* gene product) which is depicted in FIG. 18. This splice variant of the human

HKNG1 gene encodes the variant polypeptide shown in FIG. 18 (SEQ ID NO:66).

The genomic structure of the human *HKNG1* gene has been elucidated and is depicted in FIG. 3A-3R, with the *HKNG1* exons indicated in bold type, and the 5'- and 3'-untranslated regions indicated by underlining. The wild-type genomic sequence of the *HKNG1* gene is depicted in FIG. 3A-3R and SEQ ID NO:7.

Non-human homologues or orthologs mammalian orthologs, e.g., of the human *HKNG1* sequences discussed above are also provided. Specifically, a guinea pig cDNA sequence (SEQ ID NO:38), referred to herein as *gphkng1815*, encoding the full length amino acid sequence (SEQ ID NO:39) of a guinea pig HKNG1 ortholog is shown in FIG. 7. This guinea pig cDNA sequence encodes a gene product of 466 amino acid residues, as shown in FIG. 7 and in SEQ ID NO:39.

Allelic variants of this guinea pig *HKNG1* ortholog, referred to as *gphkng 7b*, *gphkng 7c*, and *gphkng 7d* (SEQ ID NOS:40, 42, and 44, respectively), are shown in FIGS. 8-10, respectively. The allelic variants *gphkng7b*, *gphkng7c*, and *gphkng7d* each encode variants of the guinea pig *gphkng1815* *HKNG1* gene product which contain deletions of 16, 92, and 93 amino acids, respectively, as shown in FIGS. 8-10, in SEQ ID NOS:41, 43, and 45, respectively, and in the sequence alignment in FIG. 14B.

Bovine *HKNG1* ortholog cDNA sequences (SEQ ID NOS: 46-48), referred to herein as *bhkng1*, *bhkng2*, and *bhkng3*, and each encoding the same bovine ortholog gene product are shown in FIGS. 11-13, respectively. The bovine *HKNG1* allelic variants encode the same gene product, *i.e.*, a 465 amino acid protein, as shown in FIGS. 11-13 and in SEQ ID NO:49.

The *HKNG1* gene nucleic acid molecules of the invention include: (a) nucleotide sequences and fragments thereof

(e.g., SEQ ID NOS: 1, 3, 5, 6, 7, 36, 37, and 65) that encode a *HKNG1* gene product (*e.g.*, SEQ ID NOS: 2, 4 and 66), as well as homologues, orthologs and allelic variants of such sequences and fragments thereof (*e.g.*, SEQ ID NOS:38, 40, 42, 44, and 46-48) which encode homologue or ortholog *HKNG1* gene products (*e.g.*, SEQ ID NOS:39, 41, 43, 45, and 49); (b) nucleotide sequences that encode one or more functional domains of a *HKNG1* gene product including, but not limited to, nucleic acid sequences that encode a signal sequence domain, or one or more clusterin domains as described in Section 5.2 below; (c) nucleotide sequences that comprise *HKNG1* gene sequences of upstream untranslated regions, intronic regions, and/or downstream untranslated regions or fragments thereof of the *HKNG1* nucleotide sequences in (a) above; (d) nucleotide sequences comprising the novel *HKNG1* sequences disclosed herein that encode mutants of the *HKNG1* gene product in which all or a part of one or more of the domains is deleted or altered, as well as fragments thereof; (e) nucleotide sequences that encode fusion proteins comprising a *HKNG1* gene product (*e.g.*, SEQ ID NO: 2, 4, 39, 41, 43, 45, 49 and 65), or a portion thereof fused to a heterologous polypeptide; and (f) nucleotide sequences (*e.g.*, primers) within the *HKNG1* gene, and chromosome 18p nucleotide sequences flanking the *HKNG1* gene which can be utilized as part of the methods of the invention for identifying and diagnosing individuals at risk for or exhibiting an *HKNG1*-mediated disorder, such as BAD, or myopia.

The *HKNG1* nucleotide sequences of the invention further include nucleotide sequences corresponding to the nucleotide sequences of (a)-(f) above wherein one or more of the exons, or fragments thereof, have been deleted. In one preferred embodiment, the *HKNG1* nucleotide sequence of the invention is

a sequence wherein the exon corresponding to exon 7 of SEQ ID NO:7, or a fragment thereof, has been deleted.

The *HKNG1* nucleotide sequences of the invention also include nucleotide sequences that have at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or more nucleotide sequence identity to the *HKNG1* nucleotide sequences of (a)-(f) above. The *HKNG1* nucleotide sequences of the invention further include nucleotide sequences that encode polypeptides having at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or higher amino acid sequence identity to the polypeptides encoded by the *HKNG1* nucleotide sequences of (a)-(f), e.g., SEQ ID NOS: 2, 4, 39, 41, 43, 45, 49, and 66 above.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical overlapping positions/total # of positions x 100%). In one embodiment, the two sequences are the same length.

The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA 87*:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA*

90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990) *J. Mol. Biol. 215*:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997) *Nucleic Acids Res.25*:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used (see http://www.ncbi.nlm.nih.gov). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, (1988) *CABIOS 4*:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

The *HKNG1* nucleotide sequences of the invention further include: (a) any nucleotide sequence that hybridizes to a *HKNG1* nucleic acid molecule of the invention under stringent conditions, *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45°C followed

by one or more washes in 0.2xSSC/0.1% SDS at about 50-65°C, or (b) under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in 6xSSC at about 45°C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68°C, or under other hybridization conditions which are apparent to those of skill in the art (see, for example, Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at pp. 6.3.1-6.3.6 and 2.10.3). Preferably the *HKNG1* nucleic acid molecule that hybridizes to the nucleotide sequence of (a) and (b), above, is one that comprises the complement of a nucleic acid molecule that encodes a *HKNG1* gene product. In a preferred embodiment, nucleic acid molecules comprising the nucleotide sequences of (a) and (b), above, encode gene products, *e.g.*, gene products functionally equivalent to an *HKNG1* gene product.

Functionally equivalent *HKNG1* gene products include naturally occurring *HKNG1* gene products present in the same or different species. In one embodiment, *HKNG1* gene sequences in non-human species map to chromosome regions syntenic to the human 18p chromosome location within which human *HKNG1* lies. Functionally equivalent *HKNG1* gene products also include gene products that retain at least one of the biological activities of the *HKNG1* gene products, and/or which are recognized by and bind to antibodies (polyclonal or monoclonal) directed against the *HKNG1* gene products.

Among the nucleic acid molecules of the invention are deoxyoligonucleotides ("oligos") which hybridize under highly stringent or stringent conditions to the *HKNG1* nucleic acid molecules described above. In general, for probes between 14 and 70 nucleotides in length the melting temperature (TM) is calculated using the formula:

Tm(°C)=81.5+16.6(log[monovalent cations (molar)])+0.41 (%

G+C)-(500/N) where N is the length of the probe. If the hybridization is carried out in a solution containing formamide, the melting temperature is calculated using the equation Tm(°C)=81.5+16.6(log[monovalent cations (molar)])+0.41(% G+C)-(0.61% formamide)-(500/N) where N is the length of the probe. In general, hybridization is carried out at about 20-25 degrees below Tm (for DNA-DNA hybrids) or 10-15 degrees below Tm (for RNA-DNA hybrids).

Exemplary highly stringent conditions may refer, e.g., to washing in 6xSSC/0.05% sodium pyrophosphate at 37°C (for about 14-base oligos), 48°C (for about 17-base oligos), 55°C (for about 20-base oligos), and 60°C (for about 23-base oligos).

These nucleic acid molecules may encode or act as antisense molecules, useful, for example, in *HKNG1* gene regulation, and/or as antisense primers in amplification reactions of *HKNG1* gene nucleic acid sequences. Further, such sequences may be used as part of ribozyme and/or triple helix sequences, also useful for *HKNG1* gene regulation. Still further, such molecules may be used as components of diagnostic methods whereby, for example, the presence of a particular *HKNG1* allele involved in a *HKNG1*-related disorder, e.g., a neuropsychiatric disorder, such as BAD, may be detected.

Fragments of the *HKNG1* nucleic acid molecules can be at least 10 nucleotides in length. In alternative embodiments, the fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, or more contiguous nucleotides in length. Alternatively, the fragments can comprise sequences that encode at least 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450 or more contiguous amino acid residues of the *HKNG1* gene products. Fragments of the *HKNG1* nucleic acid molecules can also refer to *HKNG1* exons or introns, and, further, can refer to portions of *HKNG1* coding regions that

encode domains (e.g., clusterin domains) of *HKNG1* gene products.

The *HKNG1* nucleotide sequences of the invention can be readily obtained, for example, by standard sequencing and the sequence provided herein.

As will be appreciated by those skilled in the art, DNA sequence polymorphisms of a *HKNG1* gene will exist within a population of individual organisms (*e.g.*, within a human population). Such polymorphisms may exist, for example, among individuals within a population due to natural allelic variation. Such polymorphisms include ones that lead to changes in amino acid sequence. An allele is one of a group of genes which occur alternatively at a given genetic locus.

As used herein, the phrase "allelic variant" refers to a nucleotide sequence which occurs at a given locus or to a gene product encoded by that nucleotide sequence. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of a given gene. Alternative alleles can be identified by sequencing the gene of interest in a number of different individuals. This can be readily carried out by using hybridization probes to identify the same genetic locus in a variety of individuals. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a polypeptide of the invention. The term can further include nucleic acid molecules comprising upstream and/or exon/intron sequences and sructure.

With respect to *HKNG1* allelic variants, any and all such nucleotide variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation of the *HKNG1* gene are intended to be within the scope of the present invention. Such allelic variants include, but are not limited to, ones that do not alter the functional activity of the *HKNG1* gene product. Variants include, but are not limited to, variants comprising the

polymorphisms summarized in FIGS. 5A-B and a variant which encodes a full length *HKNG1* polypeptide comprising a substitution of a lysine amino acid at amino acid residue 202 of the *HKNG1* polypeptide shown in FIG. 1A-1B and SEQ ID NO:2 or the *HKNG1* amino acid residue 184 of the polypeptide of SEQ ID NO:4.

With respect to the cloning of additional allelic variants of the human *HKNG1* gene and homologues and orthologs from other species (*e.g.*, guinea pig, cow, mouse), the isolated *HKNG1* gene sequences disclosed herein may be labeled and used to screen a cDNA library constructed from mRNA obtained from appropriate cells or tissues (*e.g.*, brain or retinal tissues) derived from the organism (*e.g.*, guinea pig, cow, and mouse) of interest. The hybridization conditions used should generally be of a lower stringency when the cDNA library is derived from an organism different from the type of organism from which the labeled sequence was derived, and can routinely be determined based on, e.g., relative relatedness of the target and refernce organisms.

Alternatively, the labeled fragment may be used t o screen a genomic library derived from the organism of interest, again, using appropriately stringent conditions. Appropriate stringency conditions are well known to those of skill in the art as discussed above, and will vary predictably depending on the specific organisms from which the library and the labeled sequences are derived. For guidance regarding such conditions see, for example, Sambrook, *et al.*, 1989, Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Press, N.Y.; and Ausubel, *et al.*, 1989-1999, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y., both of which are incorporated herein by reference in their entirety.

Further, a *HKNG1* gene allelic variant may be isolated from, for example, human nucleic acid, by performing PCR using two degenerate oligonucleotide primer pools designed on the basis of amino acid sequences within the *HKNG1* gene product disclosed herein. The template for the reaction may be cDNA obtained by reverse transcription of mRNA prepared from, for example, human or non-human cell lines or tissue known or suspected to express a wild type or mutant *HKNG1* gene allele (such as, for example, brain cells, including brain cells from individuals having BAD). In one embodiment, the allelic variant is isolated from an individual who has a *HKNG1*-mediated disorder. Such variants are described in the examples below.

The PCR product may be subcloned and sequenced to ensure that the amplified sequences represent the sequences of a *HKNG1* gene nucleic acid sequence. The PCR fragment may then be used to isolate a full length cDNA clone by a variety of methods. For example, the amplified fragment may be labeled and used to screen a bacteriophage cDNA library. Alternatively, the labeled fragment may be used to isolate genomic clones via the screening of a genomic library.

PCR technology may also be utilized to isolate full length cDNA sequences. For example, RNA may be isolated, following standard procedures, from an appropriate cellular or tissue source (*i.e.*, one known, or suspected, to express the *HKNG1* gene, such as, for example, brain tissue samples obtained through biopsy or post-mortem). A reverse transcription reaction may be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming of first strand synthesis. The resulting RNA/DNA hybrid may then be "tailed" with guanines using a standard terminal transferase reaction, the hybrid may be digested with RNAase H, and second strand synthesis may then be primed with a poly-C primer. Thus, cDNA sequences upstream of the amplified fragment may easily

be isolated. For a review of cloning strategies that may be used, see *e.g.*, Sambrook *et al.*, 1989, *supra*, or Ausubel et al., supra.

A cDNA of an allelic, e.g., mutant, variant of the *HKNG1* gene may be isolated, for example, by using PCR, a technique that is well known to those of skill in the art. In this case, the first cDNA strand may be synthesized by hybridizing an oligo-dT oligonucleotide to mRNA isolated from tissue known or suspected to be expressed in an individual putatively carrying a mutant *HKNG1* allele, and by extending the new strand with reverse transcriptase. The second strand of the cDNA is then synthesized using an oligonucleotide that hybridizes specifically to the 5' end of the normal gene. Using these two primers, the product is then amplified via PCR, cloned into a suitable vector, and subjected to DNA sequence analysis through methods well known to those of skill in the art. By comparing the DNA sequence of the mutant *HKNG1* allele to that of the normal *HKNG1* allele, the mutation(s) responsible for the loss or alteration of function of the mutant *HKNG1* gene product can be ascertained.

Alternatively, a genomic library can be constructed using DNA obtained from an individual suspected of or known to carry a mutant *HKNG1* allele, or a cDNA library can be constructed using RNA from a tissue known, or suspected, to express a mutant *HKNG1* allele. An unimpaired *HKNG1* gene or any suitable fragment thereof may then be labeled and used as a probe to identify the corresponding mutant *HKNG1* allele in such libraries. Clones containing the mutant *HKNG1* gene sequences may then be purified and subjected to sequence analysis according to methods well known to those of skill in the art.

Additionally, an expression library can be constructed utilizing cDNA synthesized from, for example, RNA isolated from a tissue known, or suspected, to express a mutant *HKNG1* allele in an individual suspected of or known to carry such a

mutant allele. In this manner, gene products made by the putatively mutant tissue may be expressed and screened using standard antibody screening techniques in conjunction with antibodies raised against the normal *HKNG1* gene product, as described, below, in Section 5.3. (For screening techniques, see, for example, Harlow and Lane, eds., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.)

In cases where a *HKNG1* mutation results in an expressed gene product with altered function (*e.g.*, as a result of a missense or a frameshift mutation), a polyclonal set of anti-*HKNG1* gene product antibodies are likely to cross-react with the mutant *HKNG1* gene product. Library clones detected via their reaction with such labeled antibodies can be purified and subjected to sequence analysis according to methods well known to those of skill in the art.

HKNG1 mutations or polymorphisms can further be detected using PCR amplification techniques. Primers can routinely be designed to amplify overlapping regions of the whole *HKNG1* sequence including the promoter regulating region. In one embodiment, primers are designed to cover the exon-intron boundaries such that, coding regions can be scanned for mutations. Exemplary primers for analyzing *HKNG1* exons are provided in Table 1, of Section 5.6, below.

The invention also includes nucleic acid molecules, preferably DNA molecules, that are the complements of the nucleotide sequences of the preceding paragraphs.

In certain embodiments, the nucleic acid molecules of the invention are present as part of nucleic acid molecules comprising nucleic acid sequences that do not contain heterologous (e.g., cloning vector or expression vector) sequences. In other embodiments, the nucleic acid molecules of the invention further comprise vector sequences, e.g., cloning vectors or expression vectors.

5.2. PROTEIN PRODUCTS OF THE *HKNG1* GENE

HKNG1 gene products or peptide fragments thereof, can be prepared for a variety of uses. For example, such gene products, or peptide fragments thereof, can be used for the generation of antibodies, in diagnostic assays, or for the identification of other cellular or extracellular gene products involved in the regulation of *HKNG1*-mediated disorders, e.g., neuropsychiatric disorders, such as BAD.

The *HKNG1* gene products of the invention include, but are not limited to, human *HKNG1* gene products, *e.g.*, polypeptides comprising the amino acid sequences depicted in FIGS. 1A-1B, 2A-2B, 17, and 18 (*i.e.*, SEQ ID NOS:2, 4, 51, and 66). The *HKNG1* gene products of the invention also include non-human, *e.g.*, mammalian (such as bovine or guinea pig), *HKNG1* gene products. These include, but are not limited to, polypeptides comprising the amino acid sequences depicted in FIGS. 7-13 (*i.e.*, SEQ ID NOS:39, 41, 43, 45, and 49).

HKNG1 gene product, sometimes referred to herein as an "*HKNG1* protein" or "*HKNG1* polypeptide," includes those gene products encoded by the *HKNG1* gene sequences depicted in FIGS. 1A-1B, 2A-2B, 7-13, 17, and 18, as well as gene products encoded by other human allelic variants and non-human variants of *HKNG1* that can be identified by the methods herein described. Among such *HKNG1* gene product variants are gene products comprising *HKNG1* amino acid residues encoded by the polymorphisms depicted in FIGS. 5A and 5B. Such gene product variants also include a variant of the *HKNG1* gene product depicted in FIG. 1 (SEQ ID NO:2) wherein the amino acid residue Lys202 is mutated to a glutamic acid residue. Such *HKNG1* gene product variants also include a variant of the *HKNG1* gene product depicted in FIG. 2 (SEQ ID NO:4)

wherein the amino acid residue Lys184 is mutated to a glutamic acid residue.

In addition, *HKNG1* gene products may include proteins that represent functionally equivalent gene products. Functionally equivalent gene products may include, for example, gene products encoded by one of the *HKNG1* nucleic acid molecules described in Section 5.1, above. In preferred embodiments, such functionally equivalent *HKNG1* gene products are naturally occuring gene products. Functionally equivalent *HKNG1* gene products also include gene products that retain at least one of the biological activities of the *HKNG1* gene products described above, and/or which are recognized by and bind to antibodies (polyclonal or monoclonal) directed against *HKNG1* gene products.

Equivalent *HKNG1* gene product may contain deletions, including internal deletions, additions, including additions yielding fusion proteins, or substitutions of amino acid residues within and/or adjacent to the amino acid sequence encoded by the *HKNG1* gene sequences described, above, in Section 5.1. Generally, deletions will be deletions of single amino acid residues, or deletions of no more than about 2, 3, 4, 5, 10 or 20 amino acid residues, either contiguous or non-contiguous. Generally, additions or substitutions, other than additions that yield fusion proteins, will be additions or substitutions of single amino acid residues, or additions or substitutions of no more than about 2, 3, 4, 5, 10 or 20 amino acid residues, either contiguous or non-contiguous. Preferably, these modifications result in a "silent" change, in that the change produces a *HKNG1* gene product with the same activity as the *HKNG1* gene product depicted in FIG. 1A-1B, 2A-2B, 7-13, or 17.

Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues

involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Alternatively, where alteration of function is desired, addition(s), deletion(s) or non-conservative alterations can produce altered, including reduced-activity, *HKNG1* gene products. Such alterations can, for example, alter one or more of the biological functions of the *HKNG1* gene product. Further, such alterations can be selected so as to generate *HKNG1* gene products that are better suited for expression, scale up, etc. in the host cells chosen. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges.

As another example, altered *HKNG1* gene products can be engineered that correspond to variants of the *HKNG1* gene product associated with *HKNG1*-mediated neuropsychiatric disorders such as BAD. Such altered *HKNG1* gene products include, but are not limited to, *HKNG1* proteins or peptides comprising substitution of a lysine residue for the wild-type glutamic acid residue at *HKNG1* amino acid position 202 in FIG. 1A-1B (SEQ ID NO:2) or amino acid position 184 (SEQ ID NO:4) in FIG. 2A-2B.

HKNG1 protein fragments and/or HKNG1 peptides comprise at least as many contiguous amino acid residues as necessary to represent an epitope fragment (that is to be recognized by an antibody directed to the HKNG1 protein). For example, such protein fragments or peptides comprise at least about 8 contiguous HKNG1 amino acid residues from a full length HKNG1 protein. In alternate embodiments, the HKNG1 protein fragments and peptides of the invention can comprise about

10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450 or more contiguous amino acid residues of a HKNG1 protein.

Peptides and/or proteins corresponding to one or more domains of the *HKNG1* protein as well as fusion proteins in which a *HKNG1* protein, or a portion of a *HKNG1* protein such as a truncated HKNG1 protein or peptide or a *HKNG1* protein domain, is fused to an unrelated protein are also within the scope of this invention. Such proteins and peptides can be designed on the basis of the *HKNG1* nucleotide sequence disclosed in Section 5.1, above, and/or on the basis of the *HKNG1* amino acid sequence disclosed in the Section. Fusion proteins include, but are not limited to, IgFc fusions which stabilize the *HKNG1* protein or peptide and prolong half life *in vivo;* or fusions to any amino acid sequence that allows the fusion protein to be anchored to the cell membrane; or fusions to an enzyme, fluorescent protein, luminescent protein, or a flag epitope protein or peptide which provides a marker function.

The HKNG1 protein, the HKNG1 protein sequences described above can include a domain which comprises a signal sequence that targets the *HKNG1* gene product for secretion. As used herein, a signal sequence includes a peptide of at least about 15 or 20 amino acid residues in length which occurs at the N-terminus of secretory and membrane-bound proteins and which contains at least about 70% hydrophobic amino acid residues such as alanine, leucine, isoleucine, phenylalanine, proline, tyrosine, tryptophan, or valine. In a preferred embodiment, a signal sequence contains at least about 10 to 40 amino acid residues, preferably about 19-34 amino acid residues, and has at least about 60-80%, more preferably 65-75%, and more preferably at least about 70% hydrophobic residues. A signal sequence serves to direct a protein containing such a sequence to a lipid bilayer.

In one embodiment, a HNKNG1 protein contains a signal sequence at about amino acids 1 to 49 of SEQ ID NO:2. In another embodiment, a *HKNG1* protein contains a signal sequence at about amino acids 30-49 of SEQ ID NO:2. In yet another embodiment, a HKNG1 protein contains a signal sequence at about amino acid residues 1 to 31 of SEQ ID NO:4. In yet another embodiment, a *HKNG1* protein contains a signal sequence at about amino acids 12-31 of SEQ ID NO:4. The signal sequence is cleaved during processing of the mature protein.

A signal sequence of a polypeptide of the invention can be used to facilitate secretion and isolation of the secreted protein or other proteins of interest. Signal sequences are typically characterized by a core of hydrophobic amino acids which are generally cleaved from the mature protein during secretion in one or more cleavage events. Such signal peptides contain processing sites that allow cleavage of the signal sequence from the mature proteins as they pass through the secretory pathway. Thus, the invention pertains to the described *HKNG1* polypeptides having a signal sequence (that is, "immature" polypeptides), as well as to the *HKNG1* signal sequences themselves and to the *HNKG1* polypeptides in the absence of a signal sequence (*i.e.*, the "mature" *HKNG1* cleavage products). It is to be understood that *HKNG1* polypeptides of the invention can further comprise polypeptides comprising any signal sequence having characteristics as described above and a mature *HKNG1* polypeptide sequence.

In one embodiment, a nucleic acid sequence encoding a signal sequence of the invention can be operably linked in an expression vector to a protein of interest, such as a protein which is ordinarily not secreted or is otherwise difficult to isolate. The signal sequence directs secretion of the protein, such as from a eukaryotic host into which the expression vector is transformed, and the signal sequence is

subsequently or concurrently cleaved. The protein can then be readily purified from the extracellular medium by art recognized methods. Alternatively, the signal sequence can be linked to the protein of interest using a sequence which facilitates purification, such as with a GST domain.

The HKNG1 protein sequences described above can also include one or more domains which comprise a clusterin domain, *i.e.*, domains which are identical to or substantially homologous to (i.e., 65%, 75%, 80%, 85%, 90%, 95% or more identical to) the domain corresponding to amino acid residues 134 to 160 or amino acid residues 334 to 362 of SEQ ID NO:2, or to the domain corresponding to amino acid residues 105-131 or amino acid residues 305-333 of SEQ ID No:39, or to the domain corresponding to amino acid residues 105-131 or amino acid residues 304-332 of SEQ ID NO:49. Preferably, such domains comprise cysteine amino acid residues at positions corresponding to conserved cysteine residues of the clusterin domains of SEQ ID NOS: 2, 39 or 49.

In particular, HKNG1 protein sequences described above can also include one or more domains which comprise a conserved cysteine domain. Such a domain corresponds, for example, to the domain of cysteines corresponding to Cys134, Cys145, Cys148, Cys158 and Cys160; or to Cys 334, Cys344, Cys351, Cys354, and Cys362 of SEQ ID NO:2. In an alternative embodiment, a conserved cystein domain corresponds to one or more of the domains of SEQ ID NO:39 which comprises Cys105, Cys116, Cys119, Cys124, and Cys131; or Cys305, Cys315, Cys322, Cys325, and Cys333. In yet another alternative embodiment, a conserved cysteine domain corresponds to one or more of the domains of SEQ ID NO:49 which comprises Cys105, Cys116, Cys119, Cys124, and Cys131; or Cys314, Cys321, Cys324, and Cys332.

Finally, the HKNG1 proteins of the invention also include HKNG1 protein sequences wherein domains encoded by one or more exons of the cDNA sequence, or fragments thereof, have been deleted. In one particularly preferred embodiment,

the HKNG1 proteins of the invention are proteins in which the domain(s) corresponding the those domains encoded by exon 7 of SEQ ID NO:7, or fragments thereof, have been deleted.

The *HKNG1* polypeptides of the invention can further comprise posttranslational modifications, including, but not limited to glycosylations, acetylations, and myrisalations.

The *HKNG1* gene products, peptide fragments thereof and fusion proteins thereof, may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the *HKNG1* gene products, polypeptides, peptides, fusion peptide and fusion polypeptides of the invention by expressing nucleic acid containing *HKNG1* gene sequences are described herein. Methods that are well known to those skilled in the art can be used to construct expression vectors containing *HKNG1* gene product coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook, *et al.*, 1989, *supra*, and Ausubel, *et al.*, 1989, *supra*. Alternatively, RNA capable of encoding *HKNG1* gene product sequences may be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in "Oligonucleotide Synthesis", 1984, Gait, ed., IRL Press, Oxford.

A variety of host-expression vector systems may be utilized to express the *HKNG1* gene product coding sequences of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells that may, when transformed or transfected with the appropriate nucleotide coding sequences, exhibit the *HKNG1* gene product of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E.*

coli, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing *HKNG1* gene product coding sequences; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing the *HKNG1* gene product coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the *HKNG1* gene product coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing *HKNG1* gene product coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the *HKNG1* gene product being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of *HKNG1* gene product or for raising antibodies to *HKNG1* gene product, for example, vectors that direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, EMBO J. 2:1791), in which the *HKNG1* gene product coding sequence may be ligated individually into the vector in frame with the *lacZ* coding region so that a fusion protein is produced; pIN vectors (Inouye and Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke and Schuster, 1989, J. Biol.

Chem. 264:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica*, nuclear polyhidrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The *HKNG1* gene product coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of *HKNG1* gene product coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (*e.g.*, see Smith, *et al.*, 1983, J. Virol. 46:584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the *HKNG1* gene product coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable

of expressing *HKNG1* gene product in infected hosts. (*e.g.*, See Logan and Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:3655-3659). Specific initiation signals may also be required for efficient translation of inserted *HKNG1* gene product coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire *HKNG1* gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the *HKNG1* gene coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner, *et al.*, 1987, Methods in Enzymol. 153:516-544).

In addition, a host cell strain may be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such

mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, and WI38.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express the *HKNG1* gene product may be engineered. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines that express the *HKNG1* gene product. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the *HKNG1* gene product.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, *et al.*, 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska and Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, *et al.*, 1980, Cell 22:817) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler, *et al.*, 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, *et al.*, 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan and Berg, 1981, Proc. Natl.

Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, 1981, J. Mol. Biol. 150:1); and hygro, which confers resistance to hygromycin (Santerre, *et al.*, 1984, Gene 30:147).

Alternatively, the expression characteristics of an endogenous *HKNG1* gene within a cell line or microorganism may be modified by inserting a heterologous DNA regulatory element into the genome of a stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous *HKNG1* gene. For example, an endogenous *HKNG1* gene which is normally "transcriptionally silent", *i.e.*, an *HKNG1* gene which is normally not expressed, or is expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, a transcriptionally silent, endogenous *HKNG1* gene may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with an endogenous *HKNG1* gene, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described *e.g.*, in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991.

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht, *et al.* allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:8972-8976). In this system, the gene of interest is subcloned

into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+}·nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

The *HKNG1* gene products can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, sheep, cows, and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate *HKNG1* transgenic animals. The term "transgenic," as used herein, refers to animals expressing *HKNG1* gene sequences from a different species (*e.g.*, mice expressing huma *HKNG1* gene sequences), as well as animals that have been genetically engineered to overexpress endogenous (*i.e.*, same species) *HKNG1* sequences or animals that have been genetically engineered to no longer express endogenous *HKNG1* gene sequences (*i.e.*, "knock-out" animals), and their progeny.

Any technique known in the art may be used to introduce a *HKNG1* gene transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to pronuclear microinjection (Hoppe and Wagner, 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten, *et al.*, 1985, Proc. Natl. Acad. Sci., USA 82:6148-6152); gene targeting in embryonic stem cells (Thompson, *et al.*, 1989, Cell 56:313-321); electroporation of embryos (Lo, 1983, Mol. Cell. Biol. 3:1803-1814); and sperm-mediated gene transfer (Lavitrano et *al.*, 1989, Cell 57:717-723) (For a review of such techniques, see Gordon, 1989, Transgenic Animals, Intl. Rev. Cytol. 115, 171-229)

Any technique known in the art may be used to produce transgenic animal clones containing a *HKNG1* transgene, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal or adult cells induced to quiescence (Campbell, *et al.*, 1996, Nature 380:64-66; Wilmut, *et al.*, Nature 385:810-813).

The present invention provides for transgenic animals that carry a *HKNG1* transgene in all their cells, as well as animals that carry the transgene in some, but not all their cells, *i.e.*, mosaic animals. The transgene may be integrated as a single transgene or in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko *et al.* (Lasko, *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:6232-6236). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the *HKNG1* transgene be integrated into the chromosomal site of the endogenous *HKNG1* gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous *HKNG1* gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous *HKNG1* gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous *HKNG1* gene in only that cell type, by following, for example, the teaching of Gu, *et al.* (Gu, *et al.*, 1994, Science 265, 103-106). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant *HKNG1* gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to assay whether integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques that include but are not limited to Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and RT-PCR (reverse transcriptase PCR). Samples of *HKNG1* gene-expressing tissue, may also be evaluated immunocytochemically using antibodies specific for the *HKNG1* transgene product.

HKNG1 proteins can be used, e.g., to treat CNS-related disorders, e.g., neuropsychiatric disorders. Such *HKNG1* gene products include but are not limited to soluble derivatives such as peptides or polypeptides corresponding to one or more domains of the *HKNG1* gene product, particularly *HKNG1* gene products, that are modified such that they are deleted for one or more hydrophobic domains. Alternatively, antibodies to the *HKNG1* protein or anti-idiotypic antibodies that mimic the *HKNG1* gene product (including Fab fragments), antagonists or agonists can be used to treat neuropsychiatric disorders involving *HKNG1*. In yet another approach, nucleotide constructs encoding such *HKNG1* gene products can be used to genetically engineer host cells to express such *HKNG1* gene products in vivo; these genetically engineered cells can function as "bioreactors" in the body delivering a continuous supply of *HKNG1* gene product, *HKNG1* peptides, soluble *HKNG1* polypeptides.

5.3. ANTIBODIES TO *HKNG1* GENE PRODUCTS

Described herein are methods for the production of antibodies capable of specifically recognizing one or more

HKNG1 gene product epitopes or epitopes of conserved variants or peptide fragments of the *HKNG1* gene products. Further, antibodies that specifically recognize mutant forms of *HKNG1*, are encompassed by the invention. The terms "specifically bind" and "specifically recognize" refer to antibodies that bind to *HKNG1* gene product epitopes at a higher affinity than they bind to non-*HKNG1* (e.g., random) epitopes.

Such antibodies may include, but are not limited to, polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above, including the polyclonal and monoclonal antibodies described in Section 12 below. Such antibodies may be used, for example, in the detection of a *HKNG1* gene product in an biological sample and may, therefore, be utilized as part of a diagnostic or prognostic technique whereby patients may be tested for abnormal levels of *HKNG1* gene products, and/or for the presence of abnormal forms of such gene products. Such antibodies may also be utilized in conjunction with, for example, compound screening schemes, as described, below, in Section 5.8, for the evaluation of the effect of test compounds on *HKNG1* gene product levels and/or activity. Additionally, such antibodies can be used in conjunction with the gene therapy techniques described, below, in Section 5.9.2 to, for example, evaluate the normal and/or engineered *HKNG1*-expressing cells prior to their introduction into the patient.

Anti-*HKNG1* gene product antibodies may additionally be used in methods for inhibiting abnormal *HKNG1* gene product activity. Thus, such antibodies may, therefore, be utilized as part of treatment methods for a *HKNG1*-mediated neuropsychiatric disorder, such as BAD or schizophrenia.

For the production of antibodies against a *HKNG1* gene product, various host animals may be immunized by injection with a *HKNG1* gene product, or a portion thereof. Such host animals may include, but are not limited to rabbits, mice, and rats, to name but a few. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen, such as a *HKNG1* gene product, or an antigenic functional derivative thereof. For the production of polyclonal antibodies, host animals such as those described above, may be immunized by injection with *HKNG1* gene product supplemented with adjuvants as also described above.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, may be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique of Kohler and Milstein, (1975, Nature 256:495-497; and U.S. Patent No. 4,376,110), the human B-cell hybridoma technique (Kosbor *et al.*, 1983, Immunology Today 4:72; Cole *et al.*, 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole *et al.*, 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in*

vitro or *in vivo*. Production of high titers of mAbs *in vivo* makes this the presently preferred method of production.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison, *et al*., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger, *et al*., 1984, Nature 312:604-608; Takeda, *et al*., 1985, Nature, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. (See, *e.g.*, Cabilly et al., U.S. Patent No. 4,816,567; and Boss et al., U.S. Patent No. 4,816397, which are incorporated herein by reference in their entirety.)

In addition, techniques have been developed for the production of humanized antibodies. (See, *e.g.*, Queen, U.S. Patent No. 5,585,089, which is incorporated herein by reference in its entirety.) An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions, referred to as complementarily determining regions (CDRs). The extent of the framework region and CDRs have been precisely defined (see, "Sequences of Proteins of Immunological Interest", Kabat, E. et al., U.S. Department of Health and Human Services (1983)). Briefly, humanized antibodies are antibody molecules from non-human species having one or more CDRs from the non-human species and a framework region from a human immunoglobulin molecule.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; Bird, 1988, Science 242:423-426; Huston, *et al*., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward, *et al*., 1989, Nature 334:544-546) can be adapted to produce single chain

antibodies against *HKNG1* gene products. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragments, which can be produced by pepsin digestion of the antibody molecule and the Fab fragments, which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed (Huse, *et al.*, 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

5.4. USES OF *HKNG1* GENE SEQUENCES GENE PRODUCTS, AND ANTIBODIES

Described herein are various applications of *HKNG1* gene sequences, *HKNG1* gene products, including peptide fragments and fusion proteins thereof, and of antibodies directed against *HKNG1* gene products and peptide fragments thereof. Such applications include, for example, mapping of chromosome 18p, prognostic and diagnostic evaluation of *HKNG1*-mediated disorders, including CNS-related disorders, e.g., neuropsychiatric disorders, such as BAD or schizophrenia, modulation of *HKNG1*-related processes, and the identification of subjects with a predisposition to such disorders, as described, below, in Section 5.5.

Additionally, such applications include methods for the treatment of a *HKNG1*-mediated disorders, such as BAD or schizophrenia, as described, below, in Section 5.9, and for the identification of compounds that modulate the expression of the *HKNG1* gene and/or the synthesis or activity of the *HKNG1* gene product, as described below, in Section 5.8. Such compounds can include, for example, other cellular products

that are involved in such processes as mood regulation and in *HKNG1*-mediated disorders, e.g., neuropsychiatric disorders such as BAD or schizophrenia. These compounds can be used, for example, in the amelioration of *HKNG1*-mediated disorders and for the modulation of *HKNG1*-mediated processes.

Uses of the *HKNG1* gene sequences, *HKNG1* gene products, including peptide fragments and fusion proteins thereof, and of antibodies directed against *HKNG1* gene products and/or peptide fragments thereof also include prognostic and diagnostic evaluation of a *HKNG1*-mediated myopia disorder such as early-onset autosomal dominant myopia, methods for the treatment of a *HKNG1*-mediated myopia disorder, and for the identification of compound that modulate the expression of the *HKNG1* gene and/or the synthesis or activity of the *HKNG1* gene product and could therefore be used in the amelioration of a *HKNG1*-mediated myopia such as early-onset autosomal dominant myopia. Indeed, such methods are substantially identical to the methods described, below, in Sections 5.5, 5.8, and 5.9 for the diagnosis and treatment of *HKNG1*-mediated disorders.

5.5. DIAGNOSIS OF *HKNG1*-MEDIATED DISORDERS

A variety of methods can be employed for the diagnostic and prognostic evaluation of *HKNG1*-mediated disorders, e.g., neuropsychiatric disorders and for the identification of subjects having a predisposition to such disorders.

Such methods may, for example, utilize reagents such as the *HKNG1* gene nucleotide sequences described in Sections 5.1, and antibodies directed against *HKNG1* gene products, including peptide fragments thereof, as described, above, in Section 5.3. Specifically, such reagents may be used, for example, for:

(1) the detection of the presence of *HKNG1* gene mutations, or the detection of either over- or under-expression of *HKNG1* gene relative to wild-type *HKNG1* levels of expression;

(2) the detection of over- or under-abundance of *HKNG1* gene product relative to wild-type abundance of *HKNG1* gene product; and

(3) the detection of an aberrant level of *HKNG1* gene product activity relative to wild-type *HKNG1* gene product activity levels.

HKNG1 gene nucleotide sequences can, for example, be used to diagnose a *HKNG1*-mediated neuropsychiatric disorder using, for example, the techniques for *HKNG1* mutation/polymorphism detection described above in Section 5.1, and in Section 5.6 below.

Mutations at a number of different genetic loci may lead to phenotypes related to neuropsychiatric disorders. Ideally, the treatment of patients suffering from such neuropsychiatric disorder will be designed to target the particular genetic loci containing the mutation mediating the disorder. Genetic polymorphisms have been linked to differences in drug effectiveness. Thus, identification of alterations in the *HKNG1* gene, protein or gene flanking regions, can be utilized in pharmacogenetic methods to optimize therapeutic drug treatments.

In one embodiment of the present invention, therefore, alterations, *i.e.*, polymorphisms, in the *HKNG1* gene or protein encoded by genes comprising such polymorphisms, are associated with a drug or drugs' efficacy, tolerance, or toxicity, and may be used in pharmacogenomic methods to optimize therapeutic drug treatments, including therapeutic drug treatments for one of the disorders described herein, *e.g.*, *HKNG1*-mediated disorders such as schizophrenia and BAD. Such polymorphisms can be used, for example, to refine the

design of drugs by decreasing the incidence of adverse events in drug tolerance studies, *e.g.*, by identifying patient subpopulations of individuals who respond or do not respond to a particular drug therapy in efficacy studies, wherein the subpopulations have a *HKNG1* polymorphism associated with drug responsiveness or unresponsiveness. The pharmacogenomic methods of the present invention can also provide tools to identify new drug targets for designing drugs and to optimize the use of already existing drugs, *e.g.*, to increase the response rate to a drug and/or to identify and exclude non-responders from certain drug treatments (*e.g.*, individuals having a particular *HKNG1* polymorphism associated with unresponsiveness or inferior responsiveness to the drug treatment) or to decrease the undersireable side effects of certain drug treatments and/or to identify and exclude individuals with marked susceptibility to such side effects (*e.g.*, individuals having a particular *HKNG1* polymorphism associated with an undersirable side effect to the drug treatment).

In an embodiment of the present invention, polymorphisms in the *HKNG1* gene sequence or flanking this sequence, or variations in *HKNG1* gene expression, or activity, e.g., variations due to altered methylation, differential splicing, or post-translational modification of the *HKNG1* gene product, may be utilized to identify an individual having a disease or condition resulting from a *HKNG1*-mediated disorder and thus define the most effective and safest drug treatment. Assays such as those described herein may be used to identify such polymorphisms or variations in *HKNG1* gene expression or activity. Once a polymorphism in the *HKNG1* gene or in a flanking sequence in linkage disequilibrium with a disorder-causing allele, or a variation in *HKNG1* gene expression has been identified in an individual, an appropriate drug treatment can be prescribed to the individual.

For the detection of *HKNG1* gene mutations or polymorphisms, any nucleated cell can be used as a starting source for genomic nucleic acid. For the detection of *HKNG1* gene expression or *HKNG1* gene products, any cell type or tissue in which the *HKNG1* gene is expressed may be utilized.

Nucleic acid-based detection techniques are described, below, in Section 5.6. Peptide detection techniques are described, below, in Section 5.7.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits. The invention therefore also encompasses kits for detecting the presence of a polypeptide or nucleic acid of the invention in a biological sample (*i.e.*, a test sample). Such kits can be used, *e.g.*, to determine if a subject is suffering from or is at increased risk of developing a disorder associated with a disorder-causing allele, or aberrant expression or activity of a polypeptide of the invention (*e.g.*, a CNS disorder, including a neurospychiatric disorder such as BAD or schizophrenia). For example, the kit can comprise a labeled compound or agent capable of detecting the polypeptide or mRNA or DNA or *HKNG1* gene sequences, e.g., encoding the polypeptide in a biological sample. The kit can further comprise a means for determining the amount of the polypeptide or mRNA in the sample (*e.g.*, an antibody which binds the polypeptide or an oligonucleotide probe which binds to DNA or mRNA encoding the polypeptide). Kits can also include instructions for observing that the tested subject is suffering from or is at risk of developing a disorder associated with aberrant expression of the polypeptide if the amount of the polypeptide or mRNA encoding the polypeptide is above or below a normal level, or if the DNA correlates with presence of a *HKNG1* allele that causes a disorder.

For antibody-based kits, the kit can comprise, for example: (1) a first antibody (*e.g.*, attached to a solid

support) which binds to a polypeptide of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or to the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can comprise, for example: (1) an oligonucleotide (*e.g.*, a detectably labeled oligonucleotide) which hybridizes to a nucleic acid sequence encoding a polypeptide of the invention, or (2) a pair of primers, such as the primers recited in Table 1, useful for amplifying a nucleic acid molecule encoding a polypeptide of the invention.

The kit can also comprise, for example, one or more buffering agents, preservatives, or protein stabilizing agents. The kit can also comprise components necessary for detecting the detectable agent (*e.g.*, an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample. Each component of the kit is usually enclosed within an individual container and all of the various containers are within a single package along with instructions for observing whether the tested subject is suffering from or is at risk of developing a disorder associated with polymorphisms that correlate with alleles that cause *HKNG1*-related disorders, and/or aberrant levels of *HKNG1* mRNA, polypeptides or activity.

5.6. <u>DETECTION OF *HKNG1* NUCLEIC ACID MOLECULES</u>

A variety of methods can be employed to screen for the presence of *HKNG1* gene-specific mutations or polymorphisms (including polymorphisms flanking *HKNG1* gene) and to detect and/or assay levels of *HKNG1* nucleic acid sequences.

Mutations or polymorphisms within or flanking the *HKNG1* gene can be detected by utilizing a number of techniques. Nucleic acid from any nucleated cell can be used as the starting point for such assay techniques, and may be isolated

according to standard nucleic acid preparation procedures that are well known to those of skill in the art.

HKNG1 nucleic acid sequences may be used in hybridization or amplification assays of biological samples to detect abnormalities involving *HKNG1* gene structure, including point mutations, insertions, deletions, inversions, translocations and chromosomal rearrangements. Such assays may include, but are not limited to, Southern analyses, single-stranded conformational polymorphism analyses (SSCP), and PCR analyses.

Diagnostic methods for the detection of *HKNG1* gene-specific mutations or polymorphisms can involve for example, contacting and incubating nucleic acids obtained from a sample, *e.g.*, derived from a patient sample or other appropriate cellular source with one or more labeled nucleic acid reagents including recombinant DNA molecules, cloned genes or degenerate variants thereof, such as described in Section 5.1, above, under conditions favorable for the specific annealing of these reagents to their complementary sequences within or flanking the *HKNG1* gene. The diagnostic methods of the present invention further encompass contacting and incubating nucleic acids for the detection of single nucleotide mutations or polymorphisms of the *HKNG1* gene. Preferably, these nucleic acid reagent sequences within the *HKNG1* gene, or chromosome 18p nucleotide sequences flanking the *HKNG1* gene are 15 to 30 nucleotides in length.

After incubation, all non-annealed nucleic acids are removed from the nucleic acid:*HKNG1* molecule hybrid. The presence of nucleic acids that have hybridized, if any such molecules exist, is then detected. Using such a detection scheme, the nucleic acid from the cell type or tissue of interest can be immobilized, for example, to a solid support such as a membrane, or a plastic surface such as that on a microtiter plate or polystyrene beads. In this case, after incubation, non-annealed, labeled nucleic acid reagents of

the type described in Section 5.1 are easily removed. Detection of the remaining, annealed, labeled *HKNG1* nucleic acid reagents is accomplished using standard techniques well-known to those in the art. The *HKNG1* gene sequences to which the nucleic acid reagents have annealed can be compared to the annealing pattern expected from a normal *HKNG1* gene sequence in order to determine whether a *HKNG1* gene mutation is present.

In a preferred embodiment, *HKNG1* mutations or polymorphisms can be detected by using a microassay of *HKNG1* nucleic acid sequences immobilized to a substrate or "gene chip" (see, *e.g.* Cronin, et al., 1996, Human Mutation 7:244-255).

Alternative diagnostic methods for the detection of *HKNG1* gene-specific nucleic acid molecules (or *HKNG1* flanking sequences), in patient samples or other appropriate cell sources, may involve their amplification, *e.g.*, by PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Patent No. 4,683,202), followed by the analysis of the amplified molecules using techniques well known to those of skill in the art, such as, for example, those listed above. The resulting amplified sequences can be compared to those that would be expected if the nucleic acid being amplified contained only normal copies of the *HKNG1* gene in order to determine whether a *HKNG1* gene mutation or polymorphism in linkage disequilibrium with a disease-causing *HKNG1* allele exists.

Among those *HKNG1* nucleic acid sequences which are preferred for such amplification-related diagnostic screening analyses are oligonucleotide primers which amplify *HKNG1* exon sequences. The sequences of such oligonucleotide primers are, therefore, preferably derived from *HKNG1* intron sequences so that the entire exon, or coding region, can be analyzed as discussed below. Primer pairs useful for

amplification of *HKNG1* exons are preferably derived from adjacent introns. Appropriate primer pairs can be chosen such that each of the eleven *HKNG1* exons are amplified. Primers for the amplification of *HKNG1* exons can be routinely designed by one of ordinary skill in the art by utilizing the exon and intron sequences of *HKNG1* shown in Figure 3A-3R.

As an example, and not by way of limitation, Table 1, below, lists primers and primer pairs which can be utilized for the amplification of each of the human *HKGN1* exons one through eleven. In this table, a primer pair is listed for each exon which consists of a forward primer derived from intron sequence upstream of the exon to be amplified, and a reverse primer derived from intron sequence downstream of the exon to be amplified. For exons greater than about 300 base pairs in length, i.e., exons 4 and 7, two primer pairs are listed (marked 4a, 4b, 7a and 7b). Each of the primer pairs can be utilized, therefore, as part of a standard PCR reaction to amplify an individual *HKNG1* exon (or portion thereof). Primer sequences are depicted in a 5' to 3' orientation.

TABLE 1

	Primer Sequence		
1	cggggttggtttccacc	(SEQ ID NO:8)	forward
	gcgaggagagaaatctggg	(SEQ ID NO:9)	reverse
2	tgctcactactttgcagtgttc	(SEQ ID NO:10)	forward
	tgagatcgtgtcactgcattct	(SEQ ID NO:11)	reverse
3	gtaaatctcaaaatgttgggttaatag	(SEQ ID NO:12)	forward
	ctaactcttcttctatcattactc	(SEQ ID NO:13)	reverse
4A	tgtttattgtgtgtctgctgtg	(SEQ ID NO:14)	forward
	ggacaaccaacatgcaaacag	(SEQ ID NO:15)	reverse
4B	cccaggtgttttcaattgatgc	(SEQ ID NO:16)	foward
	agcagttttgtccttccaagtg	(SEQ ID NO:17)	reverse
5	gtgttttgtaatctgatcagatctc	(SEQ ID NO:18)	forward
	gcagtatttctggtccagatc	(SEQ ID NO:19)	reverse
6	ggtgcacatagatcatgaaatgg	(SEQ ID NO:20)	forward
	taagctgaaataggtgccttaag	(SEQ ID NO:21)	reverse
7A	tttattccatttctgtcccctac	(SEQ ID NO:22)	forward
	aaggctcagttaggtctgtatc	(SEQ ID NO:23)	reverse
7B	caggagttttaacgtcttcagac	(SEQ ID NO:24)	forward
	gactcagaaatgtctaccatttc	(SEQ ID NO:25)	reverse
8	tgtctccacttcttcaaagtgc	(SEQ ID NO:26)	forward
	caaaatgtacctgagaacttaaag	(SEQ ID NO:27)	reverse

	Primer Sequence		
9	cacctccaagtttcatggac	(SEQ ID NO:28)	forward
	caaggtatgcacgtgtcatttc	(SEQ ID NO:29)	reverse
10	gaatgtgtattgggatttagtaaac	(SEQ ID NO:30)	forward
	ttgagaattaactattcctgtcaac	(SEQ ID NO:31)	reverse
11	ccatcctggacttttactcc	(SEQ ID NO:32)	forward
	ctttcctgcaactgtgtttattg	(SEQ ID NO:33)	reverse

Each primer pair above can be used to generate an amplified sequence of about 300 base pairs. This is especially desirable in instances in which sequence analysis is performed using SSCP gel electrophoretic procedures, in that such procedures work optimally using sequences of about 300 base pairs or less.

Additional *HKNG1* nucleic acid sequences which are preferred for such amplification-related analyses are those which will detect the presence of an *HKNG1* polymorphism which differs from the *HKNG1* sequence depicted in FIG. 3A-3R. Such polymorphisms include ones which represent mutations associated with an *HKNG1*-mediated neuropsychiatric disorder, such as BAD or schizophrenia. For example, a single base mutation identified in the Example presented in Section 8, below, results in a mutant *HKNG1* gene product comprising substitution of a lysine residue for the wild-type glutamic acid residue at amino acid position 202 of the *HKNG1* amino acid sequence shown in FIG. 1A-1B (SEQ ID NO:2) or amino acid position 184 of the *HKNG1* amino acid sequence shown in FIG. 2A-2B (SEQ ID NO:4). Such polymorphisms also include ones that correlate with the presence of a HKNG1-mediated neuropsychiatric disorder, *e.g.*, polymorphisms that are in linkage disequilibrium with disorder-causing *HKNG1* alleles.

Amplification techniques are well known to those of skill in the art and can routinely be utilized in connection with primers such as those listed in Table 1 above. In general, hybridization conditions can be as follows. In general, for probes between 14 and 70 nucleotides in length the melting temperature TM is calculated using the formula: $Tm(^{\circ}C)=81.5+16.6(\log[\text{monovalent cations}])+0.41(\%G+C)-(500/N)$ where N is the length of the probe. If the hybridization is carried out in a solution containing formamide, the melting temperature is calculated using the equation $Tm(^{\circ}C)=81.5+16.6(\log[\text{monovalent cations}])+0.41(\%G+C)-(0.61\%\text{ formamide})-(500/N)$ where N is the length of the probe.

Additionally, well-known genotyping techniques can be performed to identify individuals carrying *HKNG1* gene mutations. Such techniques include, for example, the use of restriction fragment length polymorphisms (RFLPs), which involve sequence variations in one of the recognition sites for the specific restriction enzyme used.

Further, improved methods for analyzing DNA polymorphisms, which can be utilized for the identification of *HKNG1* gene-specific mutations, have been described that capitalize on the presence of variable numbers of short, tandemly repeated DNA sequences between the restriction enzyme sites. For example, Weber (U.S. Pat. No. 5,075,217) describes a DNA marker based on length polymorphisms in blocks of (dC-dA)n-(dG-dT)n short tandem repeats. The average separation of (dC-dA)n-(dG-dT)n blocks is estimated to be 30,000-60,000 bp. Markers that are so closely spaced exhibit a high frequency co-inheritance, and are extremely useful in the identification of genetic mutations, such as, for example, mutations within the *HKNG1* gene, and the diagnosis of diseases and disorders related to *HKNG1* mutations.

Also, Caskey *et al.* (U.S. Pat.No. 5,364,759) describe a DNA profiling assay for detecting short tri and tetra

nucleotide repeat sequences. The process includes extracting the DNA of interest, such as the *HKNG1* gene, amplifying the extracted DNA, and labelling the repeat sequences to form a genotypic map of the individual's DNA.

Other methods well known in the art may be used to identify single nucleotide polymorphisms (SNPs), including biallelic SNPs or biallèlic markers which have two alleles, both of which are present at a fairly high frequency in a population. Conventional techniques for detecting SNPs include, *e.g.*, conventional dot blot analysis, single stranded conformational polymorphism (SSCP) analysis (see, *e.g.*, Orita *et al.*, 1989, *Proc. Natl. Acad. Sci. USA 86*:2766-2770), denaturing gradient gel electrophoresis (DGGE), heterodulex analysis, mismatch cleavage detection, and other routine techniques well known in the art (see, *e.g.*, Sheffield *et al.*, 1989, *Proc. Natl. Acad. Sci. 86*:5855-5892; Grompe, 1993, *Nature Genetics 5*:111-117). Alternative, preferred methods of detecting and mapping SNPs involve microsequencing techniques wherein an SNP site in a target DNA is detecting by a single nucleotide primer extension reaction (see, *e.g.*, Goelet *et al.*, PCT Publication No. WO92/15712; Mundy, U.S. Patent No. 4,656,127; Vary and Diamond, U.S. Patent No. 4,851,331; Cohen *et al.*, PCT Publication No. WO91/02087; Chee *et al.*, PCT Publication No. WO95/11995; Landegren *et al.*, 1988, *Science 241*:1077-1080; Nicerson *et al.*, 1990, *Proc. Natl. Acad. Sci. U.S.A. 87*:8923-8927; Pastinen *et al.*,1997, *Genome Res. 7*:606-614; Pastinen *et al.*, 1996, *Clin. Chem. 42*:1391-1397; Jalanko *et al.*, 1992, *Clin. Chem. 38*:39-43; Shumaker *et al.*, 1996, *Hum. Mutation 7*:346-354; Caskey *et al.*, PCT Publication No. WO 95/00669).

The level of *HKNG1* gene expression can also be assayed. For example, RNA from a cell type or tissue known, or suspected, to express the *HKNG1* gene, such as brain, may be

isolated and tested utilizing hybridization or PCR techniques such as are described, above. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells to be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the *HKNG1* gene. Such analyses may reveal both quantitative and qualitative aspects of the expression pattern of the *HKNG1* gene, including activation or inactivation of *HKNG1* gene expression.

In one embodiment of such a detection scheme, a cDNA molecule is synthesized from an RNA molecule of interest (*e.g.*, by reverse transcription of the RNA molecule into cDNA). A sequence within the cDNA is then used as the template for a nucleic acid amplification reaction, such as a PCR amplification reaction, or the like. The nucleic acid reagents used as synthesis initiation reagents (*e.g.*, primers) in the reverse transcription and nucleic acid amplification steps of this method are chosen from among the *HKNG1* gene nucleic acid reagents described in Section 5.1. The preferred lengths of such nucleic acid reagents are at least 9-30 nucleotides. For detection of the amplified product, the nucleic acid amplification may be performed using radioactively or non-radioactively labeled nucleotides. Alternatively, enough amplified product may be made such that the product may be visualized by standard ethidium bromide staining or by utilizing any other suitable nucleic acid staining method.

Additionally, it is possible to perform such *HKNG1* gene expression assays "in situ", *i.e.*, directly upon tissue sections (fixed and/or frozen) of patient tissue obtained from biopsies or resections, such that no nucleic acid purification is necessary. Nucleic acid reagents such as those described in Section 5.1 may be used as probes and/or primers for such in situ procedures (see, for example, Nuovo,

G.J., 1992, "PCR In Situ Hybridization: Protocols And Applications", Raven Press, NY).

Alternatively, if a sufficient quantity of the appropriate cells can be obtained, standard Northern analysis can be performed to determine the level of mRNA expression of the *HKNG1* gene.

5.7. DETECTION OF *HKNG1* GENE PRODUCTS

Antibodies directed against unimpaired or mutant *HKNG1* gene products or conserved variants or peptide fragments thereof, which are discussed, above, in Section 5.3, may also be used as diagnostics and prognostics for a *HKNG1*-mediated disorder, e.g., a neuropsychiatric disorder such as BAD or schizophrenia. Such methods may be used to detect abnormalities in the level of *HKNG1* gene product synthesis or expression, or abnormalities in the structure, temporal expression, and/or physical location of *HKNG1* gene product. The antibodies and immunoassay methods described herein have, for example, important *in vitro* applications in assessing the efficacy of treatments for *HKNG1*-mediated disorders. Antibodies, or fragments of antibodies, such as those described below, may be used to screen potentially therapeutic compounds *in vitro* to determine their effects on *HKNG1* gene expression and *HKNG1* gene product production. The compounds that have beneficial effects on a *HKNG1*-mediated disorder, such as BAD or schizophrenia.

In vitro immunoassays may also be used, for example, to assess the efficacy of cell-based gene therapy for a *HKNG1*-mediated disorder, e.g., a neuropsychiatric disorder, such as BAD schizophrenia. Antibodies directed against *HKNG1* gene products may be used *in vitro* to determine, for example, the level of *HKNG1* gene expression achieved in cells genetically engineered to produce *HKNG1* gene product. In the case of

intracellular *HKNG1* gene products, such an assessment is done, preferably, using cell lysates or extracts. Such analysis will allow for a determination of the number of transformed cells necessary to achieve therapeutic efficacy *in vivo*, as well as optimization of the gene replacement protocol.

The tissue or cell type to be analyzed will generally include those that are known, or suspected, to express the *HKNG1* gene. The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells to be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the *HKNG1* gene.

Preferred diagnostic methods for the detection of *HKNG1* gene products, conserved variants or peptide fragments thereof, may involve, for example, immunoassays wherein the *HKNG1* gene products or conserved variants or peptide fragments are detected by their interaction with an anti-*HKNG1* gene product-specific antibody.

For example, antibodies, or fragments of antibodies, such as those described, above, in Section 5.3, may be used to quantitatively or qualitatively detect the presence of *HKNG1* gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody (see below, this Section) coupled with light microscopic, flow cytometric, or fluorimetric detection. Such techniques are especially preferred for *HKNG1* gene products that are expressed on the cell surface.

The antibodies (or fragments thereof) useful in the present invention may, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of *HKNG1* gene products, conserved variants or peptide fragments thereof. *In situ* detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody that binds to an rTs polypeptide. The antibody (or fragment) is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the *HKNG1* gene product, conserved variants or peptide fragments, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily recognize that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve *in situ* detection of a *HKNG1* gene product.

Immunoassays for *HKNG1* gene products, conserved variants, or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells in the presence of a detectably labeled antibody capable of identifying *HKNG1* gene product, conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier, such as nitrocellulose, that is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled *HKNG1* gene product specific antibody. The solid phase support may then be washed with the buffer a second time to remove unbound antibody. The amount of bound

label on the solid support may then be detected by conventional means.

By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

One of the ways in which the *HKNG1* gene product-specific antibody can be detectably labeled is by linking the same to an enzyme, such as for use in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller, A. *et al.*, 1978, J. Clin. Pathol. 31:507-520; Butler, J.E., 1981, Meth. Enzymol. 73:482-523; Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL,; Ishikawa, E. *et al.*, (eds.), 1981, Enzyme Immunoassay, Kgaku Shoin, Tokyo). The enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety that can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes that can be used to detectably label the antibody include, but are not limited to, malate

dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, α-glycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, β-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. The detection can be accomplished by colorimetric methods that employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect *HKNG1* gene products through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986). The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography.

It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, *o*-phthaldehyde and fluorescamine.

The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

5.8. SCREENING ASSAYS FOR COMPOUNDS THAT MODULATE *HKNG1* GENE ACTIVITY

The following assays are designed to identify compounds that bind to a *HKNG1* gene product, compounds that bind to proteins, or portions of proteins that interact with a *HKNG1* gene product, compounds that modulate, *e.g.*, interfere with, the interaction of a *HKNG1* gene product with proteins and compounds that modulate the activity of the *HKNG1* gene (*i.e.*, modulate the level of *HKNG1* gene expression and/or modulate the level of *HKNG1* gene product activity). Assays may additionally be utilized that identify compounds that bind to *HKNG1* gene regulatory sequences (*e.g.*, promoter sequences; see *e.g.*, Platt, 1994, J. Biol. Chem. 269, 28558-28562), and that can modulate the level of *HKNG1* gene expression. Such compounds may include, but are not limited to, small organic molecules, such as ones that are able to cross the blood-brain barrier, gain to and/or entry into an appropriate cell

and affect expression of the *HKNG1* gene or some other gene involved in a *HKNG1* regulatory pathway.

Methods for the identification of such proteins are described, below, in Section 5.8.2. Such proteins may be involved in the control and/or regulation of mood. Further, among these compounds are compounds that affect the level of *HKNG1* gene expression and/or *HKNG1* gene product activity and that can be used in the therapeutic treatment of *HKNG1*-mediated disorders, e.g., neuropsychiatric disorders such as BAD and schizophrenia as described, below, in Section 5.9.

Compounds may include, but are not limited to, peptides such as, for example, soluble peptides, including but not limited to, Ig-tailed fusion peptides, and members of random peptide libraries; (see, *e.g.*, Lam, *et al.*, 1991, Nature 354:82-84; Houghten, *et al.*, 1991, Nature 354:84-86), and combinatorial chemistry-derived molecular library made of D- and/or L- configuration amino acids, phosphopeptides (including, but not limited to members of random or partially degenerate, directed phosphopeptide libraries; see, *e.g.*, Songyang, *et al.*, 1993, Cell 72:767-778), antibodies (including, but not limited to, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, $F(ab')_2$ and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

Such compounds may further comprise compounds, in particular drugs or members of classes or families of drugs, known to ameliorate the symptoms of a *HKNG1*-mediated disorder, e.g., a neuropsychiatric disorder such as BAD or schizophrenia.

Such compounds include families of antidepressants such as lithium salts, carbamazepine, valproic acid, lysergic acid diethylamide (LSD), *p*-chlorophenylalanine, *p*-propyldopacetamide dithiocarbamate derivatives *e.g.*, FLA 63;

anti-anxiety drugs, *e.g.*, diazepam; monoamine oxidase (MAO) inhibitors, *e.g.*, iproniazid, clorgyline, phenelzine and isocarboxazid; biogenic amine uptake blockers, *e.g.*, tricyclic antidepressants such as desipramine, imipramine and amitriptyline; serotonin reuptake inhibitors *e.g.*, fluoxetine; antipsychotic drugs such as phenothiazine derivatives (*e.g.*, chlorpromazine (thorazine) and trifluopromazine)), butyrophenones (*e.g.*, haloperidol (Haldol)), thioxanthene derivatives (*e.g.*, chlorprothixene), and dibenzodiazepines (*e.g.*, clozapine); benzodiazepines; dopaminergic agonists and antagonists *e.g.*, L-DOPA, cocaine, amphetamine, α-methyl-tyrosine, reserpine, tetrabenazine, benzotropine, pargyline; noradrenergic agonists and antagonists *e.g.*, clonidine, phenoxybenzamine, phentolamine, tropolone.

Compounds identified via assays such as those described herein may be useful, for example, in elaborating the biological function of the *HKNG1* gene product and for ameliorating *HKNG1*-mediated neuropsychiatric disorders, such as BAD and schizophrenia. Assays for testing the effectiveness of compounds identified by, for example, techniques such as those described in Sections 5.8.1 - 5.8.3, are discussed, below, in Section 5.8.4.

5.8.1. *IN VITRO* SCREENING ASSAYS FOR COMPOUNDS THAT BIND TO THE *HKNG1* GENE PRODUCT

In vitro systems may be designed to identify compounds capable of binding the *HKNG1* gene products of the invention. Compounds identified may be useful, for example, in modulating the activity of unimpaired and/or mutant *HKNG1* gene products, may be useful in elaborating the biological function of the *HKNG1* gene product, may be utilized in screens for identifying compounds that disrupt normal *HKNG1*

gene product interactions, or may in themselves disrupt such interactions.

The principle of the assays used to identify compounds that bind to the *HKNG1* gene product involves preparing a reaction mixture of the *HKNG1* gene product and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected in the reaction mixture. These assays can be conducted in a variety of ways. For example, one method to conduct such an assay involves anchoring a *HKNG1* gene product or a test substance onto a solid support and detecting *HKNG1* gene product/test compound complexes formed on the solid support at the end of the reaction. In one embodiment of such a method, the *HKNG1* gene product may be anchored onto a solid support, and the test compound, which is not anchored, may be labeled, either directly or indirectly.

In practice, microtiter plates are conveniently utilized as the solid support. The anchored component may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized antibody, preferably a monoclonal antibody, specific for the protein to be immobilized may be used to anchor the protein to the solid surface. The surfaces may be prepared in advance and stored.

In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the

surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the previously non-immobilized component (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody).

Alternatively, a reaction can be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; *e.g.*, using an immobilized antibody specific for *HKNG1* gene product or the test compound to anchor any complexes formed in solution, and a labeled antibody specific for the other component of the possible complex to detect anchored complexes.

5.8.2. ASSAYS FOR PROTEINS THAT INTERACT WITH *HKNG1* GENE PRODUCTS

Any method suitable for detecting protein-protein interactions may be employed for identifying *HKNG1* gene product-protein interactions.

Among the traditional methods that may be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns. Utilizing procedures such as these allows for the identification of proteins, including intracellular proteins, that interact with *HKNG1* gene products. Once isolated, such a protein can be identified and can be used in conjunction with standard techniques, to identify proteins it interacts with. For example, at least a portion of the amino acid sequence of a protein that interacts with the *HKNG1* gene product can be ascertained using techniques well known to those of skill in the art, such as via the Edman degradation technique (see, *e.g.*, Creighton, 1983, "Proteins: Structures and Molecular Principles," W.H. Freeman & Co., N.Y., pp.34-49). The amino acid sequence obtained may be used as a guide for the

generation of oligonucleotide mixtures that can be used to screen for gene sequences encoding such proteins. Screening made be accomplished, for example, by standard hybridization or PCR techniques. Techniques for the generation of oligonucleotide mixtures and the screening are well-known. (See, *e.g.*, Ausubel, *supra*, and 1990, "PCR Protocols: A Guide to Methods and Applications," Innis, *et al.*, eds. Academic Press, Inc., New York).

Additionally, methods may be employed that result in the simultaneous identification of genes that encode a protein which interacts with a *HKNG1* gene product. These methods include, for example, probing expression libraries with labeled *HKNG1* gene product, using *HKNG1* gene product in a manner similar to the well known technique of antibody probing of λgt11 libraries.

One method that detects protein interactions *in vivo*, the two-hybrid system, is described in detail for illustration only and not by way of limitation. One version of this system has been described (Chien, *et al.*, 1991, Proc. Natl. Acad. Sci. USA, 88:9578-9582) and is commercially available from Clontech (Palo Alto, CA).

Briefly, utilizing such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the *HKNG1* gene product and the other consists of the transcription activator protein's activation domain fused to an unknown protein that is encoded by a cDNA that has been recombined into this plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (*e.g.*, HBS or *lacZ*) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene: the DNA-binding domain hybrid cannot because it does not provide activation

function and the activation domain hybrid cannot because it cannot localize to the activator's binding sites. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product.

The two-hybrid system or related methodologies may be used to screen activation domain libraries for proteins that interact with the "bait" gene product. By way of example, and not by way of limitation, *HKNG1* gene products may be used as the bait gene product. Total genomic or cDNA sequences are fused to the DNA encoding an activation domain. This library and a plasmid encoding a hybrid of a bait *HKNG1* gene product fused to the DNA-binding domain are co-transformed into a yeast reporter strain, and the resulting transformants are screened for those that express the reporter gene. For example, a bait *HKNG1* gene sequence, such as the open reading frame of the *HKNG1* gene, can be cloned into a vector such that it is translationally fused to the DNA encoding the DNA-binding domain of the GAL4 protein. These colonies are purified and the library plasmids responsible for reporter gene expression are isolated. DNA sequencing is then used to identify the proteins encoded by the library plasmids.

A cDNA library of the cell line from which proteins that interact with bait *HKNG1* gene product are to be detected can be made using methods routinely practiced in the art. According to the particular system described herein, for example, the cDNA fragments can be inserted into a vector such that they are translationally fused to the transcriptional activation domain of GAL4. Such a library can be co-transformed along with the bait *HKNG1* gene-GAL4 fusion plasmid into a yeast strain that contains a lacZ gene driven by a promoter that contains GAL4 activation sequence. A cDNA encoded protein, fused to a GAL4 transcriptional activation domain that interacts with bait *HKNG1* gene product

will reconstitute an active GAL4 protein and thereby drive expression of the HIS3 gene. Colonies that express HIS3 can be detected by their growth on petri dishes containing semi-solid agar based media lacking histidine. The cDNA can then be purified from these strains, and used to produce and isolate the bait *HKNG1* gene product-interacting protein using techniques routinely practiced in the art.

5.8.3. ASSAYS FOR COMPOUNDS THAT INTERFERE WITH OR POTENTIATE *HKNG1* GENE PRODUCT MACROMOLECULE INTERACTION

The *HKNG1* gene products may, *in vivo*, interact with one or more macromolecules, including intracellular macromolecules, such as proteins. Such macromolecules may include, but are not limited to, nucleic acid molecules and those proteins identified via methods such as those described, above, in Sections 5.8.1 - 5.8.2. For purposes of this discussion, the macromolecules are referred to herein as "binding partners". Compounds that disrupt *HKNG1* gene product binding to a binding partner may be useful in regulating the activity of the *HKNG1* gene product, especially mutant *HKNG1* gene products. Such compounds may include, but are not limited to molecules such as peptides, and the like, as described, for example, in Section 5.8.2 above.

The basic principle of an assay system used to identify compounds that interfere with or potentiate the interaction between the *HKNG1* gene product and a binding partner or partners involves preparing a reaction mixture containing the *HKNG1* gene product and the binding partner under conditions and for a time sufficient to allow the two to interact and bind, thus forming a complex. In order to test a compound for inhibitory activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound may be initially included in the reaction mixture, or may be added at a time subsequent to the addition of *HKNG1*

gene product and its binding partner. Control reaction mixtures are incubated without the test compound or with a compound which is known not to block complex formation. The formation of any complexes between the *HKNG1* gene product and the binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the *HKNG1* gene product and the binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal *HKNG1* gene product may also be compared to complex formation within reaction mixtures containing the test compound and a mutant *HKNG1* gene product. This comparison may be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal *HKNG1* gene product.

In order to test a compound for potentiating activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound may be initially included in the reaction mixture, or may be added at a time subsequent to the addition of *HKNG1* gene product and its binding partner. Control reaction mixtures are incubated without the test compound or with a compound which is known not to block complex formation. The formation of any complexes between the *HKNG1* gene product and the binding partner is then detected. Increased formation of a complex in the reaction mixture containing the test compound, but not in the control reaction, indicates that the compound enhances and therefore potentiates the interaction of the *HKNG1* gene product and the binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal *HKNG1* gene product may also be compared to complex formation within reaction mixtures containing the test compound and a mutant *HKNG1* gene product. This comparison may be important in those cases wherein it is

desirable to identify compounds that enhance interactions of mutant but not normal *HKNG1* gene product.

In alternative embodiments, the above assays may be performed using a reaction mixture containing the *HKNG1* gene product, a binding partner, and a third which disrupts or enhances *HKNG1* gene product binding to the binding partner. The reaction mixture is prepared and incubated in the presence and absence of the test compound, as described above, and the formation of any complexes between the *HKNG1* gene product and the binding partner is detected. In this embodiment, the formation of a complex in the reaction mixture containing the test compound, but not in the control reaction, indicates that the test compound interferes with the ability of the second compound to disrupt *HKNG1* gene product binding to its binding partner.

The assays for compounds that interfere with or potentiate the interaction of the *HKNG1* gene products and binding partners can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the *HKNG1* gene product or the binding partner onto a solid support and detecting complexes formed on the solid support at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with or potentiate the interaction between the *HKNG1* gene products and the binding partners, *e.g.*, by competition, can be identified by conducting the reaction in the presence of the test substance; *i.e.*, by adding the test substance to the reaction mixture prior to or simultaneously with the *HKNG1* gene product and interactive intracellular binding partner. Alternatively, test compounds that disrupt preformed complexes, *e.g.*, compounds with higher binding constants that

displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are described briefly below.

In a heterogeneous assay system, either the *HKNG1* gene product or the interactive binding partner, is anchored onto a solid surface, while the non-anchored species is labeled, either directly or indirectly. In practice, microtiter plates are conveniently utilized. The anchored species may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished simply by coating the solid surface with a solution of the *HKNG1* gene product or binding partner and drying. Alternatively, an immobilized antibody specific for the species to be anchored may be used to anchor the species to the solid surface. The surfaces may be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) and any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the

reaction products separated from unreacted components, and complexes detected; *e.g.*, using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex formation or that disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. In this approach, a preformed complex of the *HKNG1* gene product and the interactive binding partner is prepared in which either the *HKNG1* gene product or its binding partners is labeled, but the signal generated by the label is quenched due to complex formation (see, *e.g.*, U.S. Patent No. 4,109,496 by Rubenstein which utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt *HKNG1* gene product/binding partner interaction can be identified.

In another embodiment of the invention, these same techniques can be employed using peptide fragments that correspond to the binding domains of the *HKNG1* product and/or the binding partner (in cases where the binding partner is a protein), in place of one or both of the full length proteins. Any number of methods routinely practiced in the art can be used to identify and isolate the binding sites. These methods include, but are not limited to, mutagenesis of the gene encoding one of the proteins and screening for disruption of binding in a co-immunoprecipitation assay. Compensating mutations in the gene encoding the second species in the complex can then be selected. Sequence analysis of the genes encoding the respective proteins will reveal the mutations that correspond to the region of the

protein involved in interactive binding. Alternatively, one protein can be anchored to a solid surface using methods described in this Section above, and allowed to interact with and bind to its labeled binding partner, which has been treated with a proteolytic enzyme, such as trypsin. After washing, a short, labeled peptide comprising the binding domain may remain associated with the solid material, which can be isolated and identified by amino acid sequencing. Also, once the gene coding for the segments is engineered to express peptide fragments of the protein, it can then be tested for binding activity and purified or synthesized.

For example, and not by way of limitation, a *HKNG1* gene product can be anchored to a solid material as described, above, in this Section by making a GST-*HKNG1* fusion protein and allowing it to bind to glutathione agarose beads. The binding partner can be labeled with a radioactive isotope, such as ^{35}S, and cleaved with a proteolytic enzyme such as trypsin. Cleavage products can then be added to the anchored GST-*HKNG1* fusion protein and allowed to bind. After washing away unbound peptides, labeled bound material, representing the binding partner binding domain, can be eluted, purified, and analyzed for amino acid sequence by well-known methods. Peptides so identified can be produced synthetically or produced using recombinant DNA technology.

5.8.4. ASSAYS FOR IDENTIFICATION OF COMPOUNDS THAT AMELIORATE A *HKNG1*-MEDIATED DISORDER

Compounds, including but not limited to binding compounds identified via assay techniques such as those described, above, in Sections 5.8.1 - 5.8.4, can be tested for the ability to ameliorate symptoms of a *HKNG1*-mediated disorder, e.g., a CNS-related disorder, such as a neuropsychiatric disorder, including schizophrenia and bipolar affective (mood) disorders, including severe bipolar affective (mood) disorder (BP-I), bipolar affective (mood)

disorder with hypomania and major depression (BP-II), and myopia disorders

It should be noted that the assays described herein can identify compounds that affect *HKNG1* activity by either affecting *HKNG1* gene expression or by affecting the level of *HKNG1* gene product activity. For example, compounds may be identified that are involved in another step in the pathway in which the *HKNG1* gene and/or *HKNG1* gene product is involved and, by affecting this same pathway may modulate the effect of HKNG1 on the development of a *HKNG1*-mediated disorder. Such compounds can be used, e.g., as part of a therapeutic method for the treatment of the disorder.

Described below are cell-based and animal model-based assays for the identification of compounds exhibiting such an ability to ameliorate symptoms of a *HKNG1*-mediated disorder, e.g., neuropsychiatric disorder, such as BAD or schizophrenia.

First, cell-based systems can be used to identify compounds that may act to ameliorate symptoms of a *HKNG1*-mediated disorder. Such cell systems can include, for example, recombinant or non-recombinant cell, such as cell lines, that express the *HKNG1* gene.

In utilizing such cell systems, cells that express *HKNG1* may be exposed to a compound suspected of exhibiting an ability to ameliorate symptoms of a *HKNG1*-mediated disorder, e.g., a neuropsychiatric disorder, such as BAD or schizophrenia, at a sufficient concentration and for a sufficient time to elicit such an amelioration of such symptoms in the exposed cells. After exposure, the cells can be assayed to measure alterations in the expression of the *HKNG1* gene, *e.g.*, by assaying cell lysates for *HKNG1* mRNA transcripts (*e.g.*, by Northern analysis) or for *HKNG1* gene products expressed by the cell; compounds that modulate

expression of the *HKNG1* gene are good candidates as therapeutics.

In addition, animal-based systems or models for a *HKNG1*-mediated disorder, e.g., neuropsychiatric disorder, for example, transgenic mice containing a human or altered form of *HKNG1* gene, may be used to identify compounds capable of ameliorating symptoms of the disorder. Such animal models may be used as test substrates for the identification of drugs, pharmaceuticals, therapies and interventions. For example, animal models may be exposed to a compound suspected of exhibiting an ability to ameliorate symptoms, at a sufficient concentration and for a sufficient time to elicit such an amelioration of symptoms of a *HKNG1*-mediated disorder. The response of the animals to the exposure may be monitored by assessing the reversal of the symptoms of the disorder.

With regard to intervention, any treatments that reverse any aspect of symptoms of a *HKNG1*-mediated disorder, should be considered as candidates for human therapeutic intervention in such disorders. Dosages of test agents may be determined by deriving dose-response curves, as discussed in Section 5.10.1, below.

5.9. COMPOUNDS AND METHODS FOR THE TREATMENT OF *HKNG1*-MEDIATED DISORDERS

Described below are methods and compositions whereby a *HKNG1*-mediated disorder described herein, e.g., a *HKNG1*-mediated neuropsychiatric disorder, such as BAD or schizophrenia, may be treated. For example, such methods can comprise administering compounds which modulate the expression of a mammalian *HKNG1* gene and/or the synthesis or activity of a mammalian *HKNG1* gene product (*e.g.*, a recombinant *HKNG1* gene product) so symptoms of the disorder are ameliorated.

Alternatively, in those instances whereby the *HKNG1*-mediated disorders result from *HKNG1* gene mutations, such methods can comprise supplying the subject with a nucleic acid molecule encoding an unimpaired *HKNG1* gene product such that an unimpaired *HKNG1* gene product is expressed and symptoms of the disorder are ameliorated.

In another embodiment of methods for the treatment of *HKNG1*-mediated disorders resulting from *HKNG1* gene mutations, such methods can comprise supplying the subject with a cell comprising a nucleic acid molecule that encodes an unimpaired *HKNG1* gene product such that the cell expresses the unimpaired *HKNG1* gene product and symptoms of the disorder are ameliorated.

In cases in which a loss of normal *HKNG1* gene product function results in the development of a *HKNG1*-mediated disorder an increase in *HKNG1* gene product activity would facilitate progress towards an asymptomatic state in individuals exhibiting a deficient level of *HKNG1* gene expression and/or *HKNG1* gene product activity. Methods for enhancing the expression or synthesis of *HKNG1* can include, for example, methods such as those described below, in Section 5.9.2.

Alternatively, symptoms of *HKNG1*-mediated neuropsychiatric disorders, may be ameliorated by administering a compound that decreases the level of *HKNG1* gene expression and/or *HKNG1* gene product activity. Methods for inhibiting or reducing the level of *HKNG1* gene product synthesis or expression can include, for example, methods such as those described in Section 5.9.1.

In one embodiment of treatment methods, the compounds administered comprise compounds, in particular drugs, which ameliorate the symptoms of a disorder described herein as a neuropsychiatric disorder, such as BAD or schizophrenia.

Such compounds include drugs within the families of antidepressants such as lithium salts, carbamazepine, valproic acid, lysergic acid diethylamide (LSD), *p*-chlorophenylalanine, *p*-propyldopacetamide dithiocarbamate derivatives *e.g.*, FLA 63; anti-anxiety drugs, *e.g.*, diazepam; monoamine oxidase (MAO) inhibitors, *e.g.*, iproniazid, clorgyline, phenelzine and isocarboxazid; biogenic amine uptake blockers, *e.g.*, tricyclic antidepressants such as desipramine, imipramine and amitriptyline; serotonin reuptake inhibitors *e.g.*, fluoxetine; antipsychotic drugs such as phenothiazine derivatives (*e.g.*, chlorpromazine (thorazine) and trifluopromazine)), butyrophenones (*e.g.*, haloperidol (Haldol)), thioxanthene derivatives (*e.g.*, chlorprothixene), and dibenzodiazepines (*e.g.*, clozapine); benzodiazepines; dopaminergic agonists and antagonists *e.g.*, L-DOPA, cocaine, amphetamine, α-methyl-tyrosine, reserpine, tetrabenazine, benzotropine, pargyline; noradrenergic agonists and antagonists *e.g.*, clonidine, phenoxybenzamine, phentolamine, tropolone.

In another embodiment, symptoms of a disorder described herein, *e.g.*, a *HKNG1*-mediated neuropsychiatric disorder such as BAD or schizophrenia, may be ameliorated by *HKNG1* protein therapy methods, e.g., decreasing or increasing the level and/or of HKNG1-activity using the HKNG1 protein, fusion protein, and peptide sequences described in Section 5.2, above, or by the administration of proteins or protein fragments (*e.g.*, peptides) which interact with an *HKNG1* gene or gene product and thereby inhibit or potentiate its activity.

Such protein therapy may include, for example, the administration of a functional HKNG1 protein or fragments of an HKNG1 protein (*e.g.*, peptides) which represent functional HKNG1 domains.

In one embodiment, HKNG1 fragments or peptides representing a functional HKNG1 binding domain are administered to an individual such that the peptides bind to an HKNG1 binding protein, *e.g.*, an HKNG1 receptor. Such fragments or peptides may serve to inhibit HKNG1 activity in an individual by competing with, and thereby inhibiting, binding of HKNG1 to the binding protein, thereby ameliorating symptoms of a disorder described herein. Alternatively, such fragments or peptides may enhance HKNG1 activity in an individual by mimicking the function of HKNG1 *in vivo*, thereby ameliorating the symptoms of a disorder described herein.

The proteins and peptides which may be used in the methods of the invention include synthetic (*e.g.*, recombinant or chemically synthesized) proteins and peptides, as well as naturally occurring proteins and peptides. The proteins and peptides may have both naturally occurring and non-naturally occuring amino acid residues (*e.g.*, D-amino acid residues) and/or one or more non-peptide bonds (*e.g.*, imino , ester, hydrazide, semicarbazide, and azo bonds). The proteins or peptides may also contain additional chemical groups (*i.e.*, functional groups) present at the amino and/or carboxy termini, such that, for example, the stability, bioavailability, and/or inhibitory activity of the peptide is enhanced. Exemplary functional groups include hydrophobic groups (*e.g.* carbobenzoxyl, dansyl, and t-butyloxycarbonyl, groups), an acetyl group, a 9-fluorenylmethoxy-carbonyl group, and macromolecular carrier groups (*e.g.*, lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates) including peptide groups.

5.9.1. INHIBITORY ANTISENSE, RIBOZYME AND TRIPLE HELIX APPROACHES

In another embodiment, symptoms of *HKNG1*-mediated neuropsychiatric disorders may be ameliorated by decreasing

the level of *HKNG1* gene expression and/or *HKNG1* gene product activity by using *HKNG1* gene sequences in conjunction with well-known antisense, gene "knock-out," ribozyme and/or triple helix methods to decrease the level of *HKNG1* gene expression. Among the compounds that may exhibit the ability to modulate the activity, expression or synthesis of the *HKNG1* gene, including the ability to ameliorate the symptoms of a *HKNG1*-mediated neuropsychiatric disorder, such as BAD or schizophrenia, are antisense, ribozyme, and triple helix molecules. Such molecules may be designed to reduce or inhibit either unimpaired, or if appropriate, mutant target gene activity. Techniques for the production and use of such molecules are well known to those of skill in the art.

Antisense RNA and DNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. Antisense approaches involve the design of oligonucleotides that are complementary to a target gene mRNA. The antisense oligonucleotides will bind to the complementary target gene mRNA transcripts and prevent translation. Absolute complementarily, although preferred, is not required.

A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarily to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarily and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

In one embodiment, oligonucleotides complementary to non-coding regions of the *HKNG1* gene could be used in an antisense approach to inhibit translation of endogenous *HKNG1* mRNA. Antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger, *et al.*, 1989,

Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre, *et al.*, 1987, Proc. Natl. Acad. Sci. U.S.A. 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents (see, *e.g.*, Krol *et al.*, 1988, BioTechniques 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier, *et al.*, 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-0-methylribonucleotide (Inoue, *et al.*, 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue, *et al.*, 1987, FEBS Lett. 215:327-330).

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.*, by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein, *et al.* (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin, *et al.*, 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

While antisense nucleotides complementary to the target gene coding region sequence could be used, those complementary to the transcribed, untranslated region are most preferred.

Antisense molecules should be delivered to cells that express the target gene *in vivo*. A number of methods have been developed for delivering antisense DNA or RNA to cells; *e.g.*, antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (*e.g.*, antisense linked to peptides or antibodies that specifically bind receptors or antigens

expressed on the target cell surface) can be administered systemically.

A preferred approach to achieve intracellular concentrations of the antisense sufficient to suppress translation of endogenous mRNAs utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous target gene transcripts and thereby prevent translation of the target gene mRNA. For example, a vector can be introduced *e.g.*, such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, *et al.*, 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner, *et al.*, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster, *et al.*, 1982, Nature 296:39-42), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site. Alternatively, viral vectors can be used that selectively infect the desired tissue, in

which case administration may be accomplished by another route (*e.g.*, systemically).

Ribozyme molecules designed to catalytically cleave target gene mRNA transcripts can also be used to prevent translation of target gene mRNA and, therefore, expression of target gene product. (See, *e.g.*, PCT International Publication WO90/11364, published October 4, 1990; Sarver, *et al.*, 1990, Science 247, 1222-1225).

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. (For a review, see Rossi, 1994, Current Biology 4:469-471). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, *e.g.*, U.S. Patent No. 5,093,246, which is incorporated herein by reference in its entirety.

While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy target gene mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Myers, 1995, *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, VCH Publishers, New York, (see especially Figure 4, page 833) and in Haseloff and Gerlach, 1988, Nature, 334:585-591, which is incorporated herein by reference in its entirety.

Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the

target gene mRNA, *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA) and that has been extensively described by Thomas Cech and collaborators (Zaug, *et al.*, 1984, Science, 224:574-578; Zaug and Cech, 1986, Science, 231:470-475; Zaug, *et al.*, 1986, Nature, 324:429-433; published International patent application No. WO 88/04300 by University Patents Inc.; Been and Cech, 1986, Cell, 47:207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes which target eight base-pair active site sequences that are present in the target gene.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.*, for improved stability, targeting, etc.) and should be delivered to cells that express the target gene *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous target gene messages and inhibit translation. Because ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous target gene expression can also be reduced by inactivating or "knocking out" the target gene or its promoter using targeted homologous recombination (*e.g.*, see Smithies, *et al.*, 1985, Nature 317:230-234; Thomas and Capecchi, 1987, Cell 51:503-512; Thompson, *et al.*, 1989, Cell 5:313-321; each of which is incorporated by reference herein

in its entirety). For example, a mutant, non-functional target gene (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous target gene (either the coding regions or regulatory regions of the target gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express the target gene *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the target gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target gene (*e.g.*, see Thomas and Capecchi, 1987 and Thompson, 1989, *supra*). However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

Alternatively, endogenous target gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the target gene (*i.e.*, the target gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the target gene in target cells in the body. (See generally, Helene, 1991, Anticancer Drug Des., 6(6):569-584; Helene, *et al.*, 1992, Ann. N.Y. Acad. Sci., 660:27-36; and Maher, 1992, Bioassays 14(12):807-815).

Nucleic acid molecules to be used in triplex helix formation for the inhibition of transcription should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC^+ triplets across the three associated strands of the resulting triple helix. The

pyrimidine-rich molecules provide base complementarily to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

In instances wherein the antisense, ribozyme, and/or triple helix molecules described herein are utilized to inhibit mutant gene expression, it is possible that the technique may so efficiently reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles that the possibility may arise wherein the concentration of normal target gene product present may be lower than is necessary for a normal phenotype. In such cases, to ensure that substantially normal levels of target gene activity are maintained, therefore, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity may, be introduced into cells via gene therapy methods such as those described, below, in Section 5.9.2 that do not contain sequences susceptible to whatever antisense, ribozyme, or triple helix treatments are being utilized. Alternatively, in instances whereby the target gene encodes an extracellular protein, it may be preferable to co-administer normal target gene protein in order to maintain the requisite level of target gene activity.

Anti-sense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules, as discussed above. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

5.9.2. GENE REPLACEMENT THERAPY

HKNG1 gene nucleic acid sequences, described above in Section 5.1, can be utilized for transferring recombinant *HKNG1* nucleic acid sequences to cells and expressing said sequences in recipient cells. Such techniques can be used, for example, in marking cells or for the treatment of a *HKNG1*-mediated neuropsychiatric disorder. Such treatment can be in the form of gene replacement therapy. Specifically, one or more copies of a normal *HKNG1* gene or a portion of the *HKNG1* gene that directs the production of a *HKNG1* gene product exhibiting normal *HKNG1* gene function, may be inserted into the appropriate cells within a patient, using vectors that include, but are not limited to adenovirus, adeno-associated virus, and retrovirus vectors, in addition to other particles that introduce DNA into cells, such as liposomes.

Because the *HKNG1* gene is expressed in the brain, such gene replacement therapy techniques should be capable of

delivering *HKNG1* gene sequences to these cell types within patients. Thus, in one embodiment, techniques that are well known to those of skill in the art (see, *e.g.*, PCT Publication No. WO89/10134, published April 25, 1988) can be used to enable *HKNG1* gene sequences to cross the blood-brain barrier readily and to deliver the sequences to cells in the brain. With respect to delivery that is capable of crossing the blood-brain barrier, viral vectors such as, for example, those described above, are preferable.

In another embodiment, techniques for delivery involve direct administration, *e.g.*, by stereotactic delivery of such *HKNG1* gene sequences to the site of the cells in which the *HKNG1* gene sequences are to be expressed.

Additional methods that may be utilized to increase the overall level of *HKNG1* gene expression and/or *HKNG1* gene product activity include using targeted homologous recombination methods, discussed in Section 5.2, above, to modify the expression characteristics of an endogenous *HKNG1* gene in a cell or microorganism by inserting a heterologous DNA regulatory element such that the inserted regulatory element is operatively linked with the endogenous *HKNG1* gene in question. Targeted homologous recombination can thus be used to activate transcription of an endogenous *HKNG1* gene that is "transcriptionally silent", *i.e.*, is not normally expressed or is normally expressed at very low levels, or to enhance the expression of an endogenous *HKNG1* gene that is normally expressed.

Further, the overall level of *HKNG1* gene expression and/or *HKNG1* gene product activity may be increased by the introduction of appropriate *HKNG1*-expressing cells, preferably autologous cells, into a patient at positions and in numbers that are sufficient to ameliorate the symptoms of

a *HKNG1*-mediated neuropsychiatric disorder. Such cells may be either recombinant or non-recombinant.

Among the cells that can be administered to increase the overall level of *HKNG1* gene expression in a patient are normal cells, preferably brain cells, that express the *HKNG1* gene. Alternatively, cells, preferably autologous cells, can be engineered to express *HKNG1* gene sequences, and may then be introduced into a patient in positions appropriate for the amelioration of the symptoms of a *HKNG1*-mediated neuropsychiatric disorder. Alternately, cells that express an unimpaired *HKNG1* gene and that are from a MHC matched individual can be utilized, and may include, for example, brain cells. The expression of the *HKNG1* gene sequences is controlled by the appropriate gene regulatory sequences to allow such expression in the necessary cell types. Such gene regulatory sequences are well known to the skilled artisan. Such cell-based gene therapy techniques are well known to those skilled in the art, see, *e.g.*, Anderson, U.S. Patent No. 5,399,349.

When the cells to be administered are non-autologous cells, they can be administered using well known techniques that prevent a host immune response against the introduced cells from developing. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Additionally, compounds, such as those identified via techniques such as those described, above, in Section 5.8, that are capable of modulating *HKNG1* gene product activity can be administered using standard techniques that are well known to those of skill in the art. In instances in which the compounds to be administered are to involve an interaction with brain cells, the administration techniques

should include well known ones that allow for a crossing of the blood-brain barrier.

5.10. PHARMACEUTICAL PREPARATIONS AND METHODS OF ADMINISTRATION

The compounds that are determined to affect *HKNG1* gene expression or gene product activity can be administered to a patient at therapeutically effective doses to treat or ameliorate a *HKNG1*-mediated disorder or modulate a *HKNG1*-related process described herein. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of such a disorder.

5.10.1. EFFECTIVE DOSE

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the

therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of antibody, protein, or polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight.

The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

5.10.2. FORMULATIONS AND USE

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral rectal or topical administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In certain embodiments, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment. This may be

achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

For topical application, the compounds may be combined with a carrier so that an effective dosage is delivered, based on the desired activity

A topical formulation for treatment of some of the eye disorders discussed *infra* (*e.g.*, myopia) consists of an effective amount of the compounds in a ophthalmologically acceptable excipient such as buffered saline, mineral oil, vegetable oils such as corn or arachis oil, petroleum jelly, Miglyol 182, alcohol solutions, or liposomes or liposome-like products. Any of these compositions may also include preservatives, antioxidants, antibiotics, immunosuppressants, and other biologically or pharmaceutically effective agents which do not exert a detrimental effect on the compound.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for

example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

6. EXAMPLE: THE *HKNG1* GENE OF CHROMOSOME 18 IS ASSOCIATED WITH THE NEUROPSYCHIATRIC DISORDER BAD

In the Example presented in this Section, studies are described that define a narrow interval of approximately 27 kb on the short arm of human chromosome 18 which is associated with the neuropsychiatric disorder BAD. The interval is demonstrated to lie within the gene referred to herein as the *HKNG1* gene.

6.1. MATERIALS AND METHODS

6.1.1. LINKAGE DISEQUILIBRIUM

Linkage disequilibrium (LD) studies were performed using DNA from a population sample of neuropsychiatric disorder (BP-I) patients. The population sample and LD techniques were as described in Escamilla *et al.*, 1996, *Am J. Med. Genet.* *67*:244-253. The present LD study took advantage of the additional population sample collection and the additional physical markers identified via the physical mapping techniques described below.

6.1.2. YEAST ARTIFICIAL CHROMOSOME (YAC) MAPPING

For physical mapping, yeast artificial chromosomes (YACs) containing human sequences were mapped to the region being analyzed based on publicly available maps (Cohen *et al.*, 1993, C.R. Acad. Sci. 316:1484-1488). The YACs were then ordered and contig reconstructed by performing standard short tag sequence (STS)-content mapping with microsatellite markers and non-polymorphic STSs available from databases that surround the genetically defined candidate region.

6.1.3. BACTERIAL ARTIFICIAL CHROMOSOME (BAC) MAPPING

STSs from the short arm of human chromosome 18 were used to screen a human BAC library (Research Genetics, Huntsville, AL). The ends of the BACs were cloned or directly sequenced. The end sequences were used to amplify the next overlapping BACs. From each BAC, additional microsatellites were identified. Specifically, random sheared libraries were prepared from overlapping BACs within the defined genetic interval. BAC DNA was sheared with a nebulizer (CIS-US Inc., Bedford, MA). Fragments in the size range of 600 to 1,000 bp were utilized for the sublibrary production. Microsatellite sequences from the sublibraries were identified by corresponding microsatellite probes. Sequences around such repeats were obtained to enable development of PCR primers for genomic DNA.

6.1.4. RADIATION HYBRID (RH) MAPPING

Standard RH mapping techniques were applied to a Stanford G3 RH mapping panel (Research Genetics, Huntsville, AL) to order all microsatellite markers and non-polymorphic STSs in the region being analyzed.

6.1.5. SAMPLE SEQUENCING

Random sheared libraries were made from all the BACs within the defined genetic region. Approximately 9,000 subclones within the approximately 340 kb region containing the BAD interval were sequenced with vector primers in order to achieve an 8-fold sequence coverage of the region. All sequences were processed through an automated sequence analysis pipeline that assessed quality, removed vector sequences and masked repetitive sequences. The resulting sequences were then compared to public DNA and protein databases using BLAST algorithms (Altschul, *et al.*, 1990, J. Molec. Biol., 215:403-410).

All sequences were contiged using Sequencher 3.0 (Gene Code Corp.) and PHRED and PHRAP (Phill Green, Washington University) into a single DNA fragment of 340 kb.

6.2. RESULTS

Genetic regions involved in bipolar affective disorder (BAD) human genes had previously been reported to map to portions of the long (18q) and short (18p) arms of human chromosome 18 (Freimer *et al.*, 1996, Neuropsychiat. Genet. 67:254-263; Freimer *et al.*, 1996, Nature Genetics 12:436-441; and McInnis *et al.*, *Proc. Natl. Acad. Scie. U.S.A. 93*:13060-13065).

High resolution physical mapping using YAC, BAC and RH techniques. In order to provide the precise order of genetic markers necessary for linkage and LD mapping, and to guide new microsatellite marker development for finer mapping, a high resolution physical map of the 18p candidate region was developed using YAC, BAC and RH techniques.

For such physical mapping, first, YACs were mapped to the chromosome 18 region being analyzed. Using the mapped YAC contig as a framework, the region from publicly available markers spanning the 18p region were also mapped and contiged with BACs. Sublibraries from the contiged BACs were constructed, from which microsatellite marker sequences were identified and sequenced.

To ensure development of an accurate physical map, the radiation hybrid (RH) mapping technique was independently applied to the region being analyzed. RH was used to order all microsatellite markers and non-polymorphic STSs in the region. Thus, the high resolution physical map ultimately constructed was obtained using data from RH mapping and STS-content mapping.

Linkage Disequilibrium. Prior to attempting to identify gene sequences, studies were performed to further narrow the neuropsychiatric disorder region. Specifically, a linkage disequilibrium (LD) analysis was performed using population

samples and techniques as described in Section 6.1, above, which took advantage of the additional physical markers identified via the physical mapping techniques described below.

Initial LD analysis narrowed the interval which associates with BAD disorders to a 340 kb region of 18p. BAC clones within this newly identified neuropsychiatric disorder region were analyzed to identify specific genes within the region. A combination of sample sequencing, cDNA selection and transcription mapping analyses were used to arrange sequences into tentative transcription units, that is, tentatively delineating the coding sequences of genes within this genomic region of interest.

Subsequent LD analyses further narrowed the BAD region of 18p to a narrow interval of approximately 27 kb. This was accomplished by identifying the maximum haplotype shared among affected individuals using additional markers. Statistical analysis of the entire 18p candidate region indicated that the 27 kb haplotype was significantly elevated in frequency among affected Costa Rican individuals (LOD = 2.2; p = 0.0005).

This newly identified narrow interval was found to map completely within one of the transcription units identified as described above. The gene corresponding to this transcription unit is referred to herein as the *HKNG1* gene. Thus, the results of the mapping analyses presented in this Section demonstrate that the *HKNG1* gene of human chromosome 18 is associated the neuropsychiatric disorder BAD.

Analysis of the BAD interval indicated that the 27 kb BAD disease-associated chromosomal interval identified in the linkage disequilibrium studies is contained within an approximately 60 kb genomic region which contains a sequence referred to as GS4642 or rod photoreceptor protein (RPP) gene (Shimizu-Matsumoto, A. et al., 1997, Invest. Ophthalmol. Vis. Sci. 38:2576-2585).

7. EXAMPLE: SEQUENCE AND CHARACTERIZATION OF THE *HKNG1* GENE

As demonstrated in the Example presented in Section 6, above, the *HKNG1* gene is involved in the neuropsychiatric disorder BAD. The results presented in this Section further characterize the *HKNG1* gene and gene product. In particular, isolation of additional cDNA clones and analyses of genomic and cDNA sequences have revealed both the full length *HKNG1* amino acid sequence and the *HKNG1* genomic intron/exon structure. In particular, the nucleotide and predicted amino acid sequence of the *HKNG1* gene identified by these analyses disclose new *HKNG1* exon sequences, including new *HKNG1* protein coding sequence, discovered herein. Further, the expression of *HKNG1* in human tissue, especially neural tissue, is characterized by Northern and in situ hybridization analysis. The results presented herein are consistent with the *HKNG1* gene being a gene which mediates neuropsychiatric disorders such as BAD.

7.1. MATERIALS AND METHODS

HKNG1 cDNA Clone Isolation: Hybridization of a human brain and kidney cDNA library was performed according to standard techniques and identified a full-length *HKNG1* cDNA clone. In addition, a *HKNG1* cDNA derived from a splice variant was isolated, as described in Section 7.2, below.

Northern Blot Analysis: Standard RNA isolation techniques and Northern blotting procedures were followed. The *HKNG1* probe utilized corresponds to the complementary sequence of base pairs 1367 to 1578 of the full length *HKNG1* cDNA sequence (SEQ ID NO. 1). Clontech multiple tissue northern blots were probed. In particular, Clontech human I, human II, human III, human fetal II, human brain II and human brain III blots were utilized for this study.

In Situ Hybridization Analysis: Standard in situ hybridization techniques were utilized. The *HKNG1* probe utilized corresponds to the complementary sequence of base pairs 910 to 1422 of the full length *HKNG1* cDNA sequence (SEQ ID NO. 1). Brains for in situ hybridization analysis were obtained from McLean Hospital (The Harvard Brain Tissue Resource Center, Belmont, MA 02178).

Other techniques: The remaining techniques described in Section 7.2, below, were performed according to standard techniques or as discussed in Section 6.1, above.

7.2. RESULTS

7.2.1. *HKNG1* Nucleotide and Amino Acid Sequence

A human brain cDNA library was screened and a full-length clone of *HKNG1* was isolated from this library, as described above. By comparing the isolated cDNA sequence to sequences in the public databases, a clone was identified which had been previously identified as GS4642, or rod photoreceptor protein (RPP) gene (GenBank Accession No. D63813; Shimizu-Matsumoto, A. et al., 1997, Invest. Ophthalmol. Vis. Sci. 38:2576-2585). Although Shimizu-Matsumoto et al. refer to GS4642 as a full-length cDNA sequence, the isolated *HKNG1* cDNA extends approximately 200 bp beyond the 5'end of the identified GS4642 clone.

Importantly, the *HKNG1* clone isolated herein reveals that, contrary to the amino acid sequence described in Shimizu-Matsumoto et al., the full length *HKNG1* amino acid sequence contains an additional 29 amino acid residues N-terminal to what had previously been identified as the full-length *RPP* (SEQ ID NO:64). The full-length *HKNG1* nucleotide sequence (SEQ ID NO: 1) and the derived amino acid sequence of the full-length *HKNG1* polypeptide (SEQ ID NO: 2) encoded by this sequence are depicted in FIG. 1A-1B.

The full-length HKNG1 polypeptide was found to contain two clusterin similarity domains: clusterin similarity

domain 1 which corresponds to amino acid residues 134 to amino acid residue 160, and clusterin similarity domain 2 which corresponds to amino acid residue 334 to amino acid residue 362. Such cluterin domains are typically characterized by five shared cysteine residues. In clusterin domain 1, these shared cysteine residues correspond to Cys 134, Cys145, Cys148, Cys158, and Cys 160. The shared cysteine residues in clusterin domain 2 correspond to the residues Cys334, Cys344, Cys351, Cys354, and Cys362.

Full-length *HKNG1* cDNA sequence was compared with the genomic contig completed by random sheared library sequencing. Exon-intron boundaries were identified manually by aligning the two sequences in Sequencher 3.0 and by observing the conservative splicing sites where the alignments ended. This sequence comparison revealed that the additional cDNA sequence discovered through isolation of the full-length *HKNG1* cDNA clone actually belongs within three *HKNG1* exons.

Prior to the isolation and analysis of *HKNG1* cDNA described herein, nine exons were predicted to be present within the corresponding genomic sequence. As discovered herein, however, the *HKNG1* gene, in contrast, actually contains 13 exons, with the new cDNA containing sequence which corresponds to a new exon 1, exon 2 and a 5' extension of what had previously been designated exon 1. Splice variants, discussed in Section 9 below, also exist which comprise additional exons 2' and 2". The genomic sequence and intron/exon structure of the *HKNG1* gene is shown in FIG. 3A-3R.

The breakdown of exons was confirmed by the perfect alignment of the cDNA sequence with the genomic sequence and by observation of expected splicing sites flanking each of the additional, newly discovered exons.

HKNG1 nucleotide sequence was used to search databases of partial sequences of cDNA clones. This search identified

a partial cDNA sequence derived from IMAGE clone R61493 having similarity to the human *HKNG1* sequence. IMAGE clone R61493 was obtained and consists of a cDNA insert, the Lafmid BA vector backbone, and DNA originating from the oligo dT primer and Hind III adaptors used in cDNA library construction. The Lafmid BA vector nucleotide sequence is available at the URL *http://image.rzpd.de/lafmida_seq.html* and descriptions of the oligo dT primer and Hind III adaptors are available in the GENBANK record corresponding to accession number R61493.

The sequence of the cDNA insert revealed that the insert was derived from an alternatively spliced *HKNG1* mRNA variant, referred to herein as *HKNG1-V1*. In particular, this *HKNG1* variant is deleted for exon 3 of the full length 13 exon *HKNG1* sequence. The nucleotide sequence of this *HKNG1* variant (SEQ ID NO:3) is depicted in FIG. 2A-B. The amino acid sequence encoded by the *HKNG1* variant (SEQ ID NO:3) is also shown in FIG. 2A-B.

Preferably therefore, the nucleic acids of the invention include nucleic acid molecules comprising the nucleotide sequence of *HKNG1-V1* or encoding the polypeptide encoded by *HKNG1-V1* in the absence of heterologous sequences (*e.g.*, cloning vector sequences such as Lafmid BA; oligo dT primer, and Hind III adaptor).

7.2.2. *HKNG1* GENE EXPRESSION

HKNG1 gene expression was examined by Northern blot analysis in various human tissues. A transcript of approximately 2 kb was detected in fetal brain, lung and kidney, and in adult brain, kidney, pancreas, prostate, testis, ovary, stomach, thyroid, spinal cord, lymph node and trachea. An approximately 1.5 kb transcript was also seen in trachea. In addition, a larger transcript of approximately 5 kb was detected in all adult neural regions tested (that is, cerebellum, cortex, medulla, spinal cord, occipital pole,

frontal lobe, temporal, putamen, amygdala, caudatte nucleus, corpus callosum, hippocampus, whole brain, substantia nigra, subthalamic nucleus and thalamus). Once again, this is in direct contrast to previous Northern analysis of the *RPP* gene, which reported that expression was limited to the retina (Shimizu-Matsumoto, A. et al., 1997, Invest. Ophthalmal. Vis. Sci. 38:2576-2585).

Analysis of *HKNG1* the tissue distribution was extended through an in situ hybridization analysis. In particular, the *HKNG1* mRNA distribution in normal human brain tissue was analyzed. The results of this analysis are depicted in FIG. 4. As summarized in FIG. 4, *HKNG1* is expressed throughout the brain, with transcripts being localized to neuronal and grey matter cell types.

Finally, expression of *HKNG1* in recombinant cells demonstrates that the *HKNG1* gene encodes a secreted polypeptide(s).

8. A MISSENSE MUTATION WITHIN *HKNG1* CORRELATES WITH BAD

The Example presented in Section 6, above, shows that the BAD disorder maps to an interval completely contained within the *HKNG1* gene of the short arm of human chromosome 18. The Example presented in Section 7, above, characterizes the *HKNG1* gene and gene products. The results presented in this Example further these studies by identifying a mutation within the coding region of a *HKNG1* allele of an individual exhibiting a BAD disorder.

Thus, the results described herein demonstrate a positive correlation between a mutation which encodes a non-wild-type *HKNG1* polypeptide and the appearance of the neuropsychiatric disorder BAD. The results presented herein, coupled with the results presented in Section 6, above,

identify *HKNG1* as a gene which mediates neuropsychiatric disorders such as BAD.

8.1. MATERIALS AND METHODS

Pairs of PCR primers that flank each exon (see TABLE 1, above) were made and used to PCR amplify genomic DNA isolated from BAD affected and normal individuals. The amplified PCR products were analyzed using SSCP gel electrophoresis or by DNA sequencing. The DNA sequences and SSCP patterns of the affected and controls were compared and variations were further analyzed.

8.2. RESULTS

In order to more definitively show that the *HKNG1* gene mediates neuropsychiatric disorders, in particular BAD, a study was conducted to explore whether a *HKNG1* mutation that correlates with BAD could be identified.

First, exon scanning was performed on all eleven exons of the *HKNG1* gene using chromosomes isolated from three affected and one normal individual from the Costa Rican population utilized for the LD studies discussed in Section 6, above. No obvious mutations correlating with BAD were found through this analysis.

Next, *HKNG1* intron and 3'-untranslated regions within the 27 kb BAD interval were scanned by sscp and/or sequencing for all variants among three affected and one normal individual from the same population. Approximately 60 variants were identified after scanning approximately two-thirds of the 27 kb genomic interval, which can be genotyped and analyzed by haplotype sharing and LD analyses, as described above, in order to identify ones which correlate with bipolar affective disorder. Fig. 5 lists selected variants identified through this study.

Exon scanning using chromosomal DNA from the general population of Costa Rica, however, successfully identified a

HKNG1 missense mutation in an individual affected with BAD who did not share the common diseased haplotype identified by the LD analysis provided above. In particular, exon scanning was done on exons 1-11 of *HKNG1* nucleic acid from 129 individuals from the general population affected with BAD.

This analysis identified a point mutation in the coding region of exon 7 not seen in non-bipolar affected disorder individuals. Specifically, the guanine corresponding to nucleotide residue 604 of SEQ ID NO:1 (or nucleotide residue 550 of SEQ ID NO:3) had mutated to an adenine. *HKNG1* protein expressed from this mutated *HKNG1* allele comprises the substitution of a lysine residue at amino acid residue 202 of SEQ ID NO:2 (or amino acid residue 184 of SEQ ID NO:4) in place of the wild-type glutamic acid residue.

Additional *HKNG1* polymorphisms relative to the *HKNG1* wild-type sequence, and which, therefore, represent *HKNG1* alleles, were identified through sequence analysis of the *HKNG1* alleles within a collection of schizophrenic patients of mixed ethnicity from the United States and within a BAD collection from the San Francisco area. These variants are depicted in FIGS. 5A and 5B, respectively. Statistical analysis indicated that there were significantly more variants in the collection of schizophrenic patients of mixed ethnicity from the United States and the San Francisco BAD and Costa Rican BAD samples than in a collection of 242 controls ($p < 0.05$).

9. EXAMPLE: IDENTIFICATION OF ADDITIONAL <u>HKNG1 SPLICE VARIANTS</u>

This example describes the isolation and identification of three novel splice variants of the human gene HKNG1. First, a novel *HKNG1* clone was isolated from a human retinal cDNA library. This clone, which completely lacks exon 7 of the full length *HKNG1* cDNA sequence, is referred to herein as *HKNG1Δ7*. Because the deletion of exon 7 from the full length

HKNG1 sequence leads to an immediate frameshift, the clone *HKNG1Δ7* encodes a truncated form of the HKNG1 protein. The *HKNG1Δ7* cDNA sequence (SEQ ID NO:65) is depicted in FIG. 18 along with the predicted amino acid sequence (SEQ ID NO:66) of the *HKNG1Δ7* gene product it encodes.

Two other novel splice variants, referred to herein as HKNG1-V2 and HKNG1-V3, were isolated and identified by using RT-PCR analysis to isolate additional HKNG1 sequences. The following primer sequences were used:

5'-AGTTGCGTCCCTCTCTGTTG-3' (SEQ ID NO:67)

5'-GCTTCATGTTCCCGCTGTTA-3' (SEQ ID NO:68)

These splice variants included additional exons between exons 2 and 3 of the full length *HKNG1* sequence (SEQ ID NO:1).

The RT-PCR product derived from HKNG1-V2 includes a novel exon referred to as "exon 2′ ", whereas the RT-PCR product derived from HKNG1-V3 includes a novel exon referred to as "exon 2″ ". The sequence of these novel exons are provided in Table 2 below. The nucleotide sequence of the HKNG1-V2 RT-PCR product containing novel exon 2′ is depicted in FIG. 6A (SEQ ID NO:36), whereas the HKNG1-V3 RT-PCR product containing novel exon 2″ is depicted in FIG. 6B (SEQ ID NO:37). Both exon 2′ and 2″ are part of the 5'-untranslated region of the HKNG1 cDNA.

TABLE 2

Exon 2'	5'-TTCCCTCCCTTTGGAACGCAGCGTGGGCACC TGCAACGCAGAGACCACTGTATCCCCGGTGCAGA ATGTAATGAGTGCCTGATACATTTGCCGAATAAA CTATTCCAAGGGTTGAACTTGCTGGAAGCAAGAG AAGCACTATTCTGG-3'	(SEQ ID NO:34)

Exon 2"	5'-ATGGAGTCTTGCTCTCGTTGCCCAGACTGGA GTGCACTGCTGCGATCTCAGCTCACTGCAACCTC TACCTCCCAGGTTCAAGCGATTCTCCTGCCTCAG CCTCTCGAGTGGCTGGGACTATAG-3'	(SEQ DI NO:35)

10. EXAMPLE: IDENTIFICATION OF HKNG1 ORTHOLOGS

This example describes the isolation and characterization of genes in other mammalian species which are orthologs to human *HKNG1*. Specifically, both guinea pig and bovine *HKNG1* sequences are described.

10.1. GUINEA PIG HKNG1 ORTHOLOGS

A guinea pig HKNG1 ortholog, referred to as *gphkng1815*, was isolated using RT-PCR. The cDNA sequence (SEQ ID NO:38) and predicted amino acid sequence (SEQ ID NO:39) are depicted in FIG. 7. Both the nucleotide and the predicted amino acid sequence of *gphkng 1815* are similar to the human HKNG1 nucleotide and amino acid sequences. Specifically, the program ALIGNv2.0 identified a 71.5% nucleotide sequence identity and a 62.8% amino acid sequence identity using standard parameters (Scoring Matrix: PAM120; GAP penalties: -12/-4).

Like the human HKNG1 polypeptide, the predicted gphkng 1815 polypeptide also contains two clusterin similarity domains, which correspond to amino acid residues 105 to 131 (clusterin domain 1), and amino acid residues 305-333 (clusterin domain 2), respectively. Both of these domains contain the five conserved cysteine residues typically associated with clusterin domains. Specifically, these conserved cysteines correspond to Cys105, Cys116, Cys119, Cys124 and Cys131 (clusterin similarity domain 1) and Cys305, Cys315, Cys322, Cys325, and Cys333 (clusterin similarity domain 1) of the gphkng 1815 polypeptide sequence.

Three allelic variants of *gphkng 1815*, referred to as *gphkng 7b*, *gphkng 7c*, and *gphkng 7d*, respectively, were also

identified by RT-PCR. Their nucleotide [SEQ ID NO:40 (*gphkng 7b*), SEQ ID NO:42 (*gphkng 7c*), and SEQ ID NO:44 (*gphkng 7d*)] and amino acid [SEQ ID NO:41 (*gphkng 7b*), SEQ ID NO:43 (*gphkng 7c*), and SEQ ID NO:45 (*gphkng 7d*)] sequences are depicted in FIGS. 8 through 10, respectively. Each of these three allelic variants contains a deletion within a region homologous to exon 7 of human *HKNG1*. The allelic variants retain the open reading frame of the gene, however, each allelic variant contains a deletion, relative to *gphkng 1815*, of 16, 92, and 93 amino acid residues, respectively.

An alignment of the predicted amino acid sequences of *gphkng1815*, *gphkng 7b*, *gphkng 7c*, and *gphkng7d* is shown in FIG. 14. An alignment of the predicted amino acid sequences of the human *HKNG1* gene product, the guinea pig HKNG1 ortholog gphkng1815, and the bovine HKNG1 ortholog described in Subsection 10.2 below are shown in FIG. 16.

10.2. BOVINE HKNG1 ORTHOLOGS

Bovine orthologs of HKNG1 were also cloned by screening a cDNA library made from pooled bovine retinal tissue using a nucleotide sequence that corresponded to the complementary sequence of base pairs 910-1422 of the full length human *HKNG1* cDNA sequence (SEQ ID NO:1) as a probe. Three independent bovine cDNA species, referred to as bhkng1, bhkng2, and bhkng3 (SEQ ID NOS: 46 to 48, respectively) were isolated. Each of these allelic variants contains several single nucleotide polymorphisms (SNPs). None of the SNPs results in an altered predicted amino acid sequence. Thus all three bovine cDNAs encodes the same predicted amino acid sequence (SEQ ID NO:49). These SNPs apparently reflect the natural allelic variation of the pooled cDNA library from which the sequences were isolated. Each of the three bovine HKNG1 allelic variants is depicted in FIGS. 11 to 13, respectively, along with the predicted amino acid sequence which they encode.

The predicted bovine HKNG1 polypeptide also contains two clusterin similarity domains, corresponding to amino acid residues 105-131 and amino acid residues 304-332, respectively, of SEQ ID NO:49. Clusterin domain 1 contains the five shared cysteine amino acid residues typically associated with this type of domain: Cys105, Cys116, Cys119, Cys124, and Cys131. Clusterin domain 2 of the bovine HKNG1 polypeptide contains four conserved cystein residues: Cys314, Cys321, Cys324, and Cys332.

11. <u>EXPRESSION OF HUMAN HKNG1 GENE PRODUCT</u>

Described in this example is the construction of expression vectors and the successful expression of recombinant human HKNG1 sequences. Expression vectors are described both for native HKNG1 and for various HKNG1 fusion proteins.

11.1. <u>EXPRESSION OF HUMAN HKNG1:FLAG</u>

A human HKNG1 flag epitope-tagged protein (HKNG1:flag) vector was constructed by PCR followed by ligation into an vector for expression in HEK 293T cells. The full open-reading frame of the full length HKNG1 cDNA sequence (SEQ ID NO:5) was PCR amplified using the following primer sequences:

5' primer	5'-TTTTTCTGAATTCGCCACCATGAAAATTA AAGCAGAGAAAAACG-3'	(SEQ ID NO:52)
3' primer	5'-TTTTTGTCGACTTATCACTTGTCGTCGTC GTCCTTGTAGTCCCAGGTTTTAAAATGTTCCT TAAAATGC-3'	(SEQ ID NO:53)

the 5' primer incorporating a Kozak sequence upstream of and including the upstream initiator methionine and the 3'primer including the nucleotide sequence encoding the flag epitope DYKDDDDK (SEQ ID NO:50) followed by a termination codon.

The sequenced DNA construct was transiently transfected into HEK 293T cells in 150 mm plates using Lipofectamine (GIBCO/BRL) according to the manufacturer's protocol. Seventy-two hours post-transfection, the serum-free conditioned medium (OptiMEM, GIBCO/BRL) was harvested and

spun and the remaining monolayer of cells was lysed using 2 mL of lysis buffer [50 mM Tris pH 8.0, 150 mM NaCl, 1% NP-40, 0.05% SDS with "Complete" protease cocktail (Boehringer Mannheim) diluted according to manufacturers instructions]. Insoluble material was pelleted before preparation of SDS-PAGE samples.

Conditioned medium was electroblotted onto a PVDF membrane (Novex) after separation by SDS-PAGE on 4-20% gradient gels and probed with an M2 anti-flag polyclonal antibody (1:500, Sigma) followed by horseradish peroxidase (HRP) conjugated sheep anti-mouse antibody (1:5000, Amersham), developed using chemiluminescent reagents (Renaissance, Dupont), and exposed to autoradiography film (Biomax MR2 film, Kodak). Flag immunoreactivity appeared as a doublet of bands that migrated by SDS-PAGE between 60 and 95 kDa as determined by Multimark molecular weight markers (Novex), demonstrating secretion of the HKNG1:Flag protein. The double band indicates at least two different species with different mobilities on SDS-PAGE. Such doublets most commonly arise with posttranslational modifications to the protein, such as glycosylation and/or proteolysis. Treatment of the PNGase F (Oxford Glycosciences) according to the manufacturer's directions resulted in a single band of increased mobility, indicating that two original bands contain N-linked carbohydrate. When run in the absence of a reducing agent, the relative mobility of the immunoreactive bands was greater than 100 kDa relative to the same markers, indicating that HKNG1:flag fusion proteins may be a disulfide linked dimer or higher oligomer.

11.2. <u>EXPRESSION OF HUMAN HKNG1-V1:FLAG</u>

A human HKNG1-V1 flag epitope-tagged protein (HKNG1-V1:flag) vector was also constructed by PCR followed by ligation into an expression vector, pMET stop. The full length open-reading frame of the HKNG1-V1 cDNA sequence (SEQ

ID NO:6) was PCR amplified using the following primer sequences:

5' primer	5'-TTTTTCTGAATTCACCATGAGGACCTGGG ACTACAGTAAC-3'	(SEQ ID NO:54)
3' primer	5'-TTTTTGTCGACTTATCACTTGTCGTCGTC GTCCTTGTAGTCCCAGGTTTTAAAATGTTCCT TAAAATGC-3'	(SEQ ID NO:53)

The 5' primer incorporated a Kozak sequence upstream of and including the upstream initiator methionine. The 3' primer included the nucleotide sequence encoding the flag epitope DYKDDDDK (SEQ ID NO:50) followed by a termination codon.

The sequenced DNA construct was transiently transfected into HEK 293T cells in 150 mm plates using Lipofectamine (GIBCO/BRL) according to the manufacturer's protocol. Seventy-two hours post-transfection, the serum-free conditioned medium (OptiMEM, GIBCO/BRL) was harvested and spun and the remaining monolayer of cells was lysed using 2 mL of lysis buffer [50 mM Tris pH 8.0, 150 mM NaCl, 1% NP-40, 0.05% SDS with "Complete" protease cocktail (Boehringer Mannheim) diluted according to manufacturers instructions]. Insoluble material was pelleted before preparation of SDS-PAGE samples.

Conditioned medium was electroblotted onto a PVDF membrane (Novex) after separation by SDS-PAGE on 4-20% gradient gels and probed with an M2 anti-flag polyclonal antibody (1:500, Sigma) followed by horseradish peroxidase (HRP) conjugated sheep anti-mouse antibody (1:5000, Amersham), developed using chemiluminescent reagents (Renaissance, Dupont), and exposed to autoradiography film (Biomax MR2 film, Kodak). Flag immunoreactivity appeared as a doublet of bands that migrated by SDS-PAGE between 60 and 95 kDa as determined by Multimark molecular weight markers (Novex), demonstrating secretion of the HKNG1:Flag protein. When run in the absence of reducing agent, the relative mobility of the immunoreactive bands was greater than 100 kDA relative to the same markers, suggesting that the HKNG1-

V1:flag fusion protein may be a disulfide linked dimer or higher oligomer.

11.3. EXPRESSION OF HUMAN HKNG1:Fc

A human HKNG1/hIgG1Fc fusion protein vector was constructed by PCR. The full-length open-reading frame of the full length HKNG1 cDNA (SEQ ID NO:5) was PCR amplified using the following primer sequences:

5' primer	5'-TTTTTCTCTCGAGACCATGAAAATTAAAG CAGAGAAAAACG-3'	(SEQ ID NO:55)
3' primer	5'-TTTTTGGATCCGCTGCTGCCCAGGTTTTA AAATGTTCCTTAAAATGC-3'	(SEQ ID NO:56)

The 5' primer incorporated a Kozak sequence before the upstream methionine to the amino acid residue before the stop codon. The 3' PCR primer contained a 3 alanine linker at the junction of HKNG1 and the human IgG1 Fc domain, which starts at residues DPE. The genomic sequence of the human IgG1 Fc domain was ligated along with the PCR product into a pCDM8 vector (Invitrogen, Carlsbad CA) for transient expression.

The sequenced DNA construct was transiently transfected into HEK 293T cells in 150 mm plates using Lipofectamine (GIBCO/BRL) according to the manufacturer's protocol. Seventy-two hours post-transfection, the serum-free conditioned medium (OptiMEM, GIBCO/BRL) was harvested and spun and the remaining monolayer of cells was lysed using 2 mL of lysis buffer [50 mM Tris pH 8.0, 150 mM NaCl, 1% NP-40, 0.05% SDS with "Complete" protease cocktail (Boehringer Mannheim) diluted according to manufacturers instructions]. Insoluble material was pelleted before preparation of SDS-PAGE samples.

Conditioned medium was electroblotted onto a PVDF membrane (Novex) after separation by SDS-PAGE on 4-20% gradient gels and probed with an M2 anti-Fc polyclonal antibody (1:500, Jackson ImmunoResearch Laboratories, Inc.) followed by horseradish peroxidase (HRP) conjugated sheep anti-mouse antibody (1:5000, Amersham), developed using

chemiluminescent reagents (Renaissance, Dupont), and exposed to autoradiography film (Biomax MR2 film, Kodak). Human IgG1 Fc immunoreactivity appeared as a doublet of bands that migrated by SDS-PAGE between 148 and 60 kDa standards of the Multimark molecular weight markers (Novex), demonstrating secretion of the HKNG1:Fc fusion protein.

11.4. EXPRESSION OF HUMAN HKNG1-V1:Fc

A human HKNG1-V1/hIgG1Fc fusion protein (HKNG1-V1:Fc) vector was also constructed by PCR. The full-length open reading frame of HKNG1-V1 cDNA (SEQ ID NO:6) was PCR amplified using the following primer sequences:

5' primer	5'-TTTTTCTCTCGAGACCATGAGGACCTGGG ACTACAGTAAC-3'	(SEQ ID NO:57)
3' primer	5'-TTTTTGGATCCGCTGCTGCCCAGGTTTTA AAATGTTCCTTAAAATGC-3'	(SEQ ID NO:56)

The 5' primer incorporated a Kozak sequence before the upstream methionine to the amino acid residue before the stop codon. The 3' PCR primer contained a 3 alanine linker at the junction of HKNG1-V1 and the human IgG1 Fc domain, which starts at residues DPE. The genomic sequence of the human IgG1 Fc domain was ligated along with the PCR product into a pCDM8 vector for transient expression.

The sequenced DNA construct was transiently transfected into HEK 293T cells in 150 mm plates using Lipofectamine (GIBCO/BRL) according to the manufacturer's protocol. Seventy-two hours post-transfection, the serum-free conditioned medium (OptiMEM, GIBCO/BRL) was harvested and spun and the remaining monolayer of cells was lysed using 2 mL of lysis buffer [50 mM Tris pH 8.0, 150 mM NaCl, 1% NP-40, 0.05% SDS with "Complete" protease cocktail (Boehringer Mannheim) diluted according to manufacturers instructions]. Insoluble material was pelleted before preparation of SDS-PAGE samples.

Conditioned medium was electroblotted onto a PVDF membrane (Novex) after separation by SDS-PAGE on 4-20%

gradient gels and probed with an anti-human Fc polyclonal antibody (1:500, Jackson ImmunoResearch Laboratories, Inc.) followed by horseradish peroxidase (HRP) conjugated sheep anti-mouse antibody (1:5000, Amersham), developed using chemiluminescent reagents (Renaissance, Dupont), and exposed to autoradiography film (Biomax MR2 film, Kodak). Human IgG1 Fc immunoreactivity appeared as a doublet of bands that migrated by SDS-PAGE between 148 and 60 kDa standards of the Multimark molecular weight markers (Novex) centered approximately between 125 and 150 kDa, demonstrating secretion mediated by the HKNG1 signal peptide.

11.5. EXPRESSION OF HUMAN HKNG1Δ7:Fc

A human HKNG1Δ7:hIgG1Fc fusion protein vector was also constructed by PCR. The sequence of the HKNG1Δ7 splice variant was amplified by PCR amplification using Exons 1 through 6 of the full length HKNG1 cDNA sequence (SEQ ID NO:1) as a template with the following primer sequences:

5' primer	5'-TTTTTCTGAATTCACCATGAAGCCGCCAC TCTTGGTG-3'	(SEQ ID NO:58)
3' primer	5'-TTTTTGGATCCGCTGCGGCCTCCGTG GTCAGGAGCTTATTTTTCACAGAGGACCAGCT AG-3'	(SEQ ID NO:59)

The 5' primer incorporated a Kozak sequence upstream of and including the upsream initiator methionine. The 3' primer included the first17 (coding) nucleotides of exon 8 followed by nucleotides encoding a 3 alanine linker.

The genomic sequence of the human IgG1 Fc domain was ligated along with the PCR product into a pCDM8 vector for transient expression.

The sequenced DNA construct was transiently transfected into HEK 293T cells in 150 mm plates using Lipofectamine (GIBCO/BRL) according to the manufacturer's protocol. Seventy-two hours post-transfection, the serum-free conditioned medium (OptiMEM, GIBCO/BRL) was harvested and spun and the remaining monolayer of cells was lysed using

2 mL of lysis buffer [50 mM Tris pH 8.0, 150 mM NaCl, 1% NP-40, 0.05% SDS with "Complete" protease cocktail (Boehringer Mannheim) diluted according to manufacturers instructions]. Insoluble material was pelleted before preparation of SDS-PAGE samples.

Conditioned medium was electroblotted onto a PVDF membrane (Novex) after separation by SDS-PAGE on 4-20% gradient gels and probed with an anti-human Fc polyclonal antibody (1:500, Jackson ImmunoResearch Laboratories) followed by horseradish peroxidase (HRP) conjugated sheep anti-mouse antibody (1:5000, Amersham), developed using chemiluminescent reagents (Renaissance, Dupont), and exposed to autoradiography film (Biomax MR2 film, Kodak). Human IgG1 Fc immunoreactivity appeared as a band that migrated by SDS-PAGE between 42 and 60 kDa relative to Multimark molecular weight markers (Novex) centered approximately between 36.5 and 55.4 kDa relative to Mark 12 molecular weight markers (Novex).

11.6. EXPRESSION OF NATIVE HUMAN HKNG1

A human HKNG1 expression vector was constructed by PCR amplification of the human HKNG1 cDNA sequence (SEQ ID NO:1) followed by ligation into an expression vector, pcDNA3.1 (Invitrogen, Carlsbad CA). The full open-reading frame of the HKNG1 cDNA sequence (SEQ ID NO:5) was PCR amplified using the following primer sequences:

5' primer	5'-TTTTTCTCTCGAGGACTACAGGACACAGC TAAATCC-3'	(SEQ ID NO:60)
3' primer	5'-TTTTTGGATCCTTATCACCAGGTTTTAAA ATGTTCCTTAAAATGC-3'	(SEQ ID NO:61)

The 5' primer incorporated a Kozak sequence upstream of and including the upstream initiator methionine. The 3' primer included a tandem pair of termination codons.

The sequenced DNA construct was transiently transfected into HEK 293T cells in 150 mm plates using Lipofectamine (GIBCO/BRL) according to the manufacturer's protocol. Seventy-two hours post-transfection, the serum-free

conditioned medium (OptiMEM, GIBCO/BRL) was harvested and spun and the remaining monolayer of cells was lysed using 2 mL of lysis buffer [50 mM Tris pH 8.0, 150 mM NaCl, 1% NP-40, 0.05% SDS with "Complete" protease cocktail (Boehringer Mannheim) diluted according to manufacturers instructions]. Insoluble material was pelleted before preparation of SDS-PAGE samples.

Conditioned medium was electroblotted onto a PVDF membrane (Novex) after separation by SDS-PAGE on 4-20% gradient gels and probed with an anti-HKNG1 polyclonal antibody (#84, 1:500) followed by horseradish peroxidase (HRP) conjugated sheep anti-mouse antibody (1:5000, Amersham), developed using chemiluminescent reagents (Renaissance, Dupont), and exposed to autoradiography film (Biomax MR2 film, Kodak). HKNG1 immunoreactivity appeared as a doublet of bands that migrated by SDS-PAGE between 60 and 95 kDa as determined by Multimark molecular weight markers (Novex).

11.7. EXPRESSION OF NATIVE HUMAN HKNG1-V1

A human HKNG1-V1 expression vector was also constructed by PCR amplification of the human HKNG1-V1 cDNA sequence (SEQ ID NO:3) followed by ligation into an expression vector, pcDNA3.1. The full open-reading frame of the HKNG1 cDNA sequence (SEQ ID NO:6) was PCR amplified using the following primer sequences:

5' primer	5'-TTTTTCTGAATTCACCATGAAGCCGCCAC TCTTGGTG-3'	(SEQ ID NO:62)
5' primer	5'-TTTTTCTCTCGAGACCATGAGGACCTGGG ACTACAGTAAC-3'	(SEQ ID NO:63)
3' primer	5'-TTTTTGGATCCTTATCACCAGGTTTTAAA ATGTTCCTTAAAATGC-3'	(SEQ ID NO:61)

The 5' primer incorporated a Kozak sequence upstream of and including the upstream initiator methionine. The 3' primer included a tandem pair of termination codons.

The sequenced DNA construct was transiently transfected into HEK 293T cells in 150 mm plates using Lipofectamine

(GIBCO/BRL) according to the manufacturer's protocol. Seventy-two hours post-transfection, the serum-free conditioned medium (OptiMEM, GIBCO/BRL) was harvested and spun and the remaining monolayer of cells was lysed using 2 mL of lysis buffer [50 mM Tris pH 8.0, 150 mM NaCl, 1% NP-40, 0.05% SDS with "Complete" protease cocktail (Boehringer Mannheim) diluted according to manufacturers instructions]. Insoluble material was pelleted before preparation of SDS-PAGE samples.

Conditioned medium was electroblotted onto a PVDF membrane (Novex) after separation by SDS-PAGE on 4-20% gradient gels and probed with an M2 anti-HKNG1 polyclonal antibody (#84, 1:500) followed by horseradish peroxidase (HRP) conjugated sheep anti-mouse antibody (1:5000, Amersham), developed using chemiluminescent reagents (Renaissance, Dupont), and exposed to autoradiography film (Biomax MR2 film, Kodak). HKNG immunoreactivity appeared as a doublet of bands that migrated by SDS-PAGE between 70 and 95 kDa as determined by Multimark molecular weight markers (Novex), demonstrating secretion mediated by the HKNG1 signal peptide.

11.8. EXPRESSION OF HUMAN HKNG:AP FUSION PROTEINS

Expression vectors were also constructed for human HKNG1 alkaline phosphatase C-terminal fusion protein (HKNG1:AP), human HKNG1-V1 alkaline phosphatase C-terminal fusion protein (HKNG1-V1:AP), and human HKNG1 alkaline phosphatase N-terminal fusion protein (AP:HKNG1).

The expression vector for human HKNG1:AP was constructed by PCR amplification followed by ligation into a vector for suitable for expression in HEK 293T cells. The full-length open-reading frame of human HKNG1 (SEQ ID NO:5) was PCR amplified using a 5' primer incorporating an EcoRI restriction site followed by a Kozak sequence prior to the upstream initiator methionine. The 3' primer included a XhoI restriction site immediately following the final codon of

HKNG1. Thus, the open reading frame of the construct includes the HKNG1 signal peptide and the full HKNG1 sequence followed by the full sequence of human placental alkaline phosphatase.

The expression vector for human HKNG1-V1:AP was constructed by PCR amplification followed by ligation into pMEAP3 vector. The full length open reading frame of human *HKNG1-V1* (SEQ ID NO:6) was PCR amplified using a 5' primer incorporating an EcoRI restriction site followed by a Kozak sequence prior to the upstream initiator methionine. The 3' primer included a XhoI restriction site immediately following the final codon of *HKNG1-V1*. Thus, the open reading frame of the construct includes the HKNG1-V1 signal and the full length HKNG1-V1 sequence followed by the full sequence of human placental alkaline phosphatase.

The expression vector for human AP:HKNG1 was constructed by PCR amplification followed by ligation into the AP-Tag3 vector reported by Cheng and Flanagan, 1994, *Cell 79*:157-168. The full-length open-reading frame of human HKNG1 (SEQ ID NO:5)was PCR amplified using a 5' primer incorporating a BamHI restriction site prior to the nucleotides encoding the first amino acids (*i*.e., APT) of the mature HKNG protein, and a 3' primer that included a XhoI restriction site immediately following the termination codon of HKNG1. Thus, the open reading frame of the complete construct includes the AP signal peptide and the full sequence of human placental alkaline phosphatase, followed by the full HKNG1 sequence.

The sequenced DNA constructs were transiently transfected in HEK 293T cells in 150 mM plates using Lipofectamine (GIBCO/BRL) according to the manufacturer's protocol. 72 hours post-transfection, the serum-free conditioned media (OptiMEM, Gibco/BRL) were harvested, spun and filtered. Alkaline phosphatase activity in the conditioned media was quantitated using an enzymatic assay kit (Phospha-Light, Tropix) according to the manufacturer's instructions. When alkaline phosphatase fusion protein

concentrations below 2 nM were observed, conditioned medium was concentrated by centrifugation using a 30 kDa cut-off membrane. Conditioned medium samples before and after concentration were analyzed by SDS-PAGE followed by Western blot using anti-human alkaline phosphatase antibodies (1:250, Genzyme) and chemiluminsecent detection. A band at 140 kDa was observed in concentrated supernatant of HKNG1:AP, HKNG1-V1:AP, and AP:HKNG1 transfections. Conditioned medium samples were adjusted to 10% fetal calf serum and stored at 4ºC.

11.9. PURIFICATION OF FLAG-TAGGED HKNG1 PROTEINS

The secreted flag-tagged proteins described in subsections 12.1 and 12.2 above were isolated by a one step purification scheme utilizing the affinity of the flag epitope to M2 anti-flag antibodies. The conditioned media was passed over an M2-biotin (Sigma)/streptavidin Poros column (2.1 x 30 mm, PE Biosystems). The column was then washed with PBS, pH 7.4, and flag-tagged protein was eluted with 200 mM glycine, pH 3.0. Fraction was neutralized with 1.0 M Tris pH 8.0. Eluted fractions with 280 nm absorbance greater than background were then analyzed on SDS-PAGE gels and by Western blot. The fractions containing flag taged protein were pooled and dialyzed in 8000 MWCO dialysis tubing against 2 changes of 4L PBS, pH 7.4 at 4ºC with constant stirring. The buffered exchanged material was then sterile filtered (0.2 µm, Millipore) and frozen at -80ºC.

11.10. PURIFICATION OF HKNG1 Fc FUSION PROTEINS

The secreted Fc fusion proteins described in Subsections 12.3-12.5 above were isolated by a one step purification scheme utilizing the affinity of the human IgG1 Fc domain to Protein A. The conditioned media was passed over a POROS A column (4.6 x 100 mm, PerSeptive Biosystems); the column was then washed with PBS, pH 7.4 and eluted with 200 mM glycine, pH 3.0. Fractions were neutralized with 1.0 M Tris pH 8.0.

A constant flow rate of 7 ml/min was maintained throughout the procedure. Eluted fractions with 280 nm absorbance greater than background were then analyzed on SDS-PAGE gels and by Western blot. The fractions containing Fc fusion protein were pooled and dialyzed in 8000 MWCO dialysis tubing against 2 changes of 4L PBS, pH 7.4 at 4ºC with constant stirring. The buffered exchanged material was then sterile filtered (0.2 μm, Millipore) and frozen at -80ºC.

12. PRODUCTION OF ANTI-HKNG1 ANTIBODIES

Described in the example presented in this Section is the production and characterization of polyclonal and monoclonal antibodies directed against HKNG1 proteins.

12.1. PRODUCTION OF POLYCLONAL ANTIBODIES

Polyclonal antisera were raised in rabbits against each of the three peptides listed in Table 3 below. Each of the peptides was derived from the HKNG1 amino acid sequence (SEQ ID NO:2) by standard techniques (see, in particular, Harlow&Lane, 1988, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, the contents of which is incorporated herein by reference in its entirety). Each of the peptides is also represented in the HKNG1-V1 polypeptide sequence (SEQ ID NO:4). Antisera was subsequently affinity purified using the peptide immunogens.

TABLE 3

Antibody	Peptide/Immunogen	a.a. residues (SEQ ID NO:2)
Antibody 84	APTWKDKTAISENLK	50-64
Antibody 85	KAIEDLPKQDK	304-314
Antibody 86	KALQHFKEHFKTW	483-495

12.2. PRODUCTION OF MONOCLONAL ANTIBODIES

Monoclonal antibodies were raised in mice by standard techniques (see, Harlow & Lane, *supra*) against the HKNG-Fc fusion protein described in Section 11.3 above. Wells were screened by ELISA for binding to the HKNG-Fc fusion protein. Those wells reacting with the Fc protein were identified by ELISA for binding to an irrelevant Fc fusion protein and discarded. HKNG-Fc specific wells were tested for their ability to immunoprecipitate HKNG-Fc and subjected to isotype analysis by standard techniques (Harlow & Lane, *supra*), and eight wells were selected for subcloning. The isotype of the subcloned monoclonal antibodies was confirmed and is presented in Table 4 below.

Based on Western blotting, immunoprecipitation and immunostaining data discussed in SubSection 12.3 below, two monoclonal antibodies (3D17 and 4N6) were selected for large scale production.

TABLE 4

Clone	Isotype
1F24	2a
1J18	2a
2O20	1
3D17	1
3D24	2a
4N6	1
4O16	2b
10C6	2a

12.3. WESTERN BLOTTING AND IMMUNOPRECIPITATION OF RECOMBINANT HKNG PROTEIN

The polyclonal antisera and all eight monoclonal antibodies described in subsection 12.1 and 12.2 above were tested for their ability to recognize recombinant HKNG1 proteins on Western blots using standard techniques (see, in

particular, Harlow & Lane, 1988, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press). Polyclonal antisera 84 and 85 and monoclonal antibodies 3D17 and 4N6 were able to recognize all forms of the mature (*i.e.*, secreted) recombinant HKNG proteins tested (*i.e.*, HKNG1:Fc, HKNG1:flag, AP:HKNG1, and native HKNG1) in Western blots.

Table 5 indicates the ability of each monoclonal antibody to immunoprecipitate recombinant HKNG1, as assessed by Western blotting of immunoprecipitates with the polyclonal antisera 84 and 85. None of the polyclonal antisera were able to immunoprecipitate recombinant HKNG1 proteins. All eight monoclonal antibodies immunoprecipitated HKNG1:Fc. Immunoprecipitation of the other recombinant HKNG1 proteins was variable.

TABLE 5

Monoclonal Antibody	Protein			
	HKNG1:Fc	HKNG1:flag	AP:HKNG1	HKNG1 (native)
IF24	+	+	+	−/+
1J18	+	−	−/+	+/+
2O20	+	−	+	−
3D17	+/+	+/+	−	+/+
3D24	+	−	−	−
4N6	+	+	+	+
4O16	+	−	−	+/+
10C6	+	−	−	+

13. CONFIRMATION OF THE HKNG N-TERMINUS AND DISULFIDE BOND STRUCTURE

The experiments described in this section provide data identifying the N-terminus of the mature secreted human HKNG

protein. The experiments also provide data identifying the disulfide bond linkages between cysteine amino acid residues in the mature, secreted protein.

Specifically, mature, secreted HKNG:flag, HKNG, and HKNG:Fc recombinant proteins were produced and purified as described in Section 11 above. The mature recombinant proteins were digested with trypsin, and the tryptic fragments were identified and sequenced using reverse-phase liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC/MS/MS). The N-terminus of all mature secreted proteins tested was unambiguously identified as APTWKDKT, which corresponds to the amino acid sequence starting at alanine 50 of the HKNG1 amino acid sequence (FIG. 1; SEQ ID NO:2) or alanine 32 of the HKNG1-V1 amino acid sequence (FIG. 2; SEQ ID NO:4). Thus, although the cDNA sequences of HKNG1 and HKNG1-V1 encode distinct amino acid sequences, the mature secreted proteins produced by these two splice variants of the human *HKNG1* gene are identical, since the alternative splicing that gives rise to HKNG1-V1 (*i.e.*, the deletion of exon 3) affects the amino acid sequence of the proteolytically cleaved signal peptide. The amino acid sequence of the mature secreted HKNG1 protein is shown in FIG. 17 (SEQ ID NO:51)

The mature secreted HKNG protein is also distinct from the RPP amino acid sequence disclosed by Shimizu-Matsumo *et al.* (1997, *Invest. Ophthalmal. Vis. Sci.* *38*:2576-2585). In particular, amino acid residues 1 to 20 of the RPP amino acid sequence disclosed in Figure 3 of Shimizu-Matsumo *et al.*, *supra*, correspond to the cleaved signal peptide of HKNG1-V1. The amino acid sequence of the mature secreted form of the *HKNG1* gene product is depicted in FIG. 17 (SEQ ID NO:51).

Disulfide bond linkages for 8 of the 13 cysteine residues in the mature, secreted HKNG protein were also identified from LC/MS/MS of peptides recovered from tryptic

digestion of the unreduced protein. In particular, the following disulfide bonded pairs of cysteines were identified (numbering refers to the HKNG1 protein shown in FIG. 1; SEQ ID NO:2):

Cys 134 to Cys 145;
Cys 148 to Cys 153;
Cys 160 to Cys 334; and
Cys 354 to Cys 362.

14. EXAMPLE: LOCALIZATION OF HKNG mRNA AND PROTEIN EXPRESSION

This example describes experiments wherein the HKNG gene product is shown to be expressed in human brain and retinal tissue. Specifically, *in situ* hybridization experiments performed using standard techniques with a probe that corresponded to the complementary sequence of base pairs 910-1422 of the full length *HKNG1* cDNA sequence (SEQ ID NO:1) detected HKNG messenger RNA in the photoreceptor layer (outer nuclear layer) of human retina in eyes obtained from the New England Eye Bank.

The polyclonal antisera and all eight monoclonal antibodies described in Section 12 above were tested for immunostaining of human retina. Polyclonal antiserum 85 and monoclonal antibodies 1F24, 4N6 and 4016 showed immunostaining of HKNG protein in the photoreceptor layer and adjacent layers of the retina. The immunostaining in these tissues with polyclonal antiserum was blocked by 85 peptide immunogen, but not by the other two peptide immunogens (*i.e.*, 84 and 86), confirming that the immunostaining was due to HKNG protein expressed in the photoreceptor layer.

The same antibodies were then used to localize HKNG protein by immunostaining in sections of human and monkey brain. HKNG protein was observed in cortical neurons in the frontal cortex. The majority of pyramidal neurons in layers IV-V were immunoreactive for HKNG protein. A subpopulation of neurons was also labeled in layers I-III. HKNG

immunoreactivity was also observed in the pyramidal cell layer of the hippocampus and in a small number of neurons in the striatum.

These data further support the fact that HKNG is, indeed, a gene which mediates neuropsychiatric disorders such as BAD. Furthermore, the fact that HKNG is also expressed in human retinal tissue suggests that the gene also plays a role in myopia conditions. Specifically, Young *et al.* (1998, *American Journal of Human Genetics 63*:109-119) report a strong linkage (LOD = 9.59) for primary myopia and secondary macular degeneration and retinal detachment in the telomeric region of human chromosome 18p. Through fine mapping analysis, this candidate region has been narrowed to a 7.6 cM haplotype flanked by markers D18S59 and D18S1138 (Young *et al., supra*). However, the marker D18S59 lies within the HKNG1 gene. This fact, coupled with the finding the HKNG is expressed in high levels in the retina, strongly suggests that the HKNG1 gene is also responsible for human myopia conditions and/or other eye related diseases such as primary myopia, secondary macular degeneration, and retinal detachment.

15. EXAMPLE: IMMATURE PROTEIN PRODUCTS OF THE HKNG1 cDNA SEQUENCES

This section describes experiments which were performed to determine which of the two putative initiator methionines encoded by both the full length *HKNG1* cDNA and the alternatively spliced *HKNG1-V1* cDNA are used in the synthesis of immature HKNG1 protein. The results indicate that both initiator methionines are used at varying levels, resulting in the production of three different forms of the immature HKNG1 protein, referred to herein as immature protein form 1 (IPF1), immature protein form 2 (IPF2), and immature protein form 3 (IPF3).

Both the full length *HKNG1* cDNA sequence shown in FIG. 1 (SEQ ID NO:1) and the alternatively spliced *HKNG1-V1* cDNA sequence shown in FIG. 2 (SEQ ID NO:3) encode predicted proteins that have methionines in close proximity to their predicted initiator methionines. The predicted protein sequence encoded by the full length *HKNG1* cDNA sequence has a second methionine at amino acid residue number 30 of the amino acid sequence depicted in FIG. 1 (SEQ ID NO:2). Thus, although FIG. 1 indicates that the full length *HKNG1* cDNA encodes the first immature form of the HKNG1 protein depicted in FIG. 1 (referred to herein as IPF1), the full length *HKNG1* cDNA may additionally encode a second immature protein form (referred to herein as IPF2), whose sequence (SEQ ID NO:64) is provided on the third line of the protein alignment depicted in FIG. 17. IPF2 is initiated at methionine 30 of the IPF1 protein sequence, and is identical to the RPP polypeptide sequence taught by Shimizu-Matsumoto *et al* (1997, *Invest. Ophthalmol. Vis. Sci. 38*:2576-2585). Likewise, the alternatively spliced *HKNG1-V1* cDNA sequence encodes the predicted immature protein form, referred to herein as IPF3, depicted in FIG. 2 (SEQ ID NO:4). However, the *HKNG1-V1* cDNA may also encoded another immature protein form, identical to IPF 2, that is initiated at methionine 12 of the IPF3 protein sequence. FIG. 17 illustrates an alignment of the three immature HKNG1 protein sequences IPF1 (second row), IPF2 (third row), and IPF3 (bottom row). As explained is Section 13 above, the mature *HKNG1* gene product secreted by cells expressing the HKNG1 constructs described in Section 11, above, is in fact the same cleaved product (SEQ ID NO:51), regardless of the immature HKNG1 protein (IPF1, IPF2, or IPF3) from which it is produced. An alignment of the mature secreted HKNG1 protein is therefore also depicted in FIG. 17 (top row).

Modified HKNG1:flag and HKNG1-V1:flag expression vectors were constructed as described in Sections 12.1 and 12.2,

respectively. However, the nucleotide sequence of full length HKNG1 was modified, using standard site directed mutagenesis techniques, so as to introduce an additional base pair between the upstream methionine (*i.e.*, met 1 in SEQ ID NO:2) and the downstream methionine (*i.e.*, met 30 in SEQ ID NO:2). The nucleotide sequence of HKNG1-V1 was likewise modified, using standard site directed mutagenesis techniques, to introduce an additional base between its upstream methionine (*i.e.*, met 1 in SEQ ID NO:4) and downstream methionine (*i.e.*, met 12 in SEQ ID NO:4). Thus, in both modified constructs, the C-terminal flag epitope tag was no longer in the same reading frame as the upstream methionine but was in frame with the downstream methionine. Consequently, exclusive translation initiation at the first methionine of a construct would lead to the production of non-flag immunoreactive proteins. However, exclusive translation initiation at the second methionine of a construct would lead to the production of flag immunoreactive proteins.

Unmodified HKNG1:flag, unmodified HKNG1-V1:flag, modified HKNG1:flag, and modified HKNG1-V1:flag constructs were transfected into cells, and their resulting gene products were harvested, blotted onto a PVDF membrane, and probed with an M2 anti-flag polyclonal antibody, and developed according to the methods described in Sections 12.1 and 12.2 above.

Flag immunoreactivity was detected in all four samples. The unmodified HKNG1:flag and HKNG1-V1:flag expression vectors produced amounts of mature secreted HKNG1:flag protein consistent with the levels detected in Sections 12.1 and 12.2 above. Further, the flag immunoreactive band detected for the modified HKNG1:flag construct was indistinguishable in intensity from the band detected for the unmodified HKNG1:flag construct, indicating that the immature HKNG1 protein produced by full length *HKNG1* cDNA is

predominantly IPF2, while IPF1 is produced by full length *HKNG1* cDNA in relatively minor amounts.

The flag immunoreactive band from the modified HKNG1-V1:flag construct had dramatically reduced intensity relative to the band from the unmodified HKNG1-V1:flag construct. Thus, *HKNG1-V1* produces primarily the immature HKNG1 protein IPF3, while the immature HKNG1 protein IPF2 is produced by *HKNG1-V1* in relatively minor amounts. These results are summarized below in Table 6.

TABLE 6

Construct	Immature Protein	Prominance
HKNG1	IPF1 (SEQ ID NO:2)	Minor
	IPF2 (SEQ ID NO:64)	Predominant
HKNG1-V1	IPF2 (SEQ ID NO:64)	Minor
	IPF3 (SEQ ID NO:4)	Predominant

Thus, the *HKNG1* gene products of the invention include gene products corresponding to the immature protein forms IPF1 and IPF3. However, preferably the *HKNG1* gene products of the invention do not include amino acid sequences consisting of the IPF2 sequence (SEQ ID NO:64).

16. REFERENCES CITED

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings.

All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes a HKNG1 gene product comprising:

(a) the amino acid sequence of SEQ ID NO:2;
(b) the amino acid sequence of SEQ ID NO:4;
(c) the amino acid sequence of SEQ ID NO:39;
(d) the amino acid sequence of SEQ ID NO:41;
(e) the amino acid sequence of SEQ ID NO:43;
(f) the amino acid sequence of SEQ ID NO:45;
(g) the amino acid sequence of SEQ ID NO:49; or
(h) the amino acid sequence of SEQ ID NO:66.

2. The isolate nucleic acid molecule of Claim 1, wherein the isolate nucleic acid molecule comprises:

(a) the nucleotide sequence of SEQ ID NO:1;
(b) the nucleotide sequence of SEQ ID NO:3;
(c) the nucleotide sequence of SEQ ID NO:7;
(d) the nucleotide sequence of SEQ ID NO:34; or
(e) the nucleotide sequence of SEQ ID NO:35.

3. The isolated nucleic acid molecule of Claim 1, wherein the isolated nucleic acid molecule comprises:

(a) the nucleotide sequence of SEQ ID NO:38;
(b) the nucleotide sequence of SEQ ID NO:40;
(c) the nucleotide sequence of SEQ ID NO:42; or
(d) the nucleotide sequence of SEQ ID NO:44.

4. The isolated nucleic acid molecule of Claim 1, wherein the isolated nucleic acid molecule comprises:

(a) the nucleotide sequence of SEQ ID NO:46;
(b) the nucleotide sequence of SEQ ID NO:47; or
(c) the nucleotide sequence of SEQ ID NO:48.

5. An isolated nucleic acid molecule consisting of a nucleotide sequence that encodes a mature HKNG1 protein having the amino acid sequence of SEQ ID NO:51.

6. An isolated nucleic acid molecule which hybridizes to the complement of the nucleic acid molecule of any one of claims 1-5 under highly stringent conditions comprising washing in 0.1xSSC/0.1% SDS at 68ºC.

7. An isolated nucleic acid molecule which hybridizes to the complement of the nucleic acid molecule of any one of claims 1-5 under stringent conditions comprising washing in 0.2xSSC/0.1% SDS at 50-65ºC.

8. The isolated nucleic acid molecule of Claim 6 or 7, wherein said isolated nucleic acid molecule encodes a functionally equivalent HKNG1 gene product.

9. A vector comprising the nucleotide sequence of any one of Claims 1-5.

10. An expression vector comprising the nucleotide sequence of any one of Claims 1-5 operatively associated with a regulatory nucleotide sequence controlling the expression of the nucleotide sequence in a host cell.

11. A host cell genetically engineered to contain the nucleotide sequence of any one of Claims 1-5.

12. A host cell genetically engineered to express the nucleotide sequence of any one of Claims 1-5 operatively associated with a regulatory nucleotide sequence controlling expression of the nucleotide sequence in said host cell.

13. An isolated polypeptide comprising the amino acid sequence of a HKNG1 gene product having:

(a) the amino acid sequence of SEQ ID NO:2;
(b) the amino acid sequence of SEQ ID NO:4;
(c) the amino acid sequence of SEQ ID NO:39;
(d) the amino acid sequence of SEQ ID NO:41;
(e) the amino acid sequence of SEQ ID NO:43;

(f) the amino acid sequence of SEQ ID NO:45; or
(g) the amino acid sequence of SEQ ID NO:49;
(h) the amino acid sequence of SEQ ID NO:66.

14. An isolated polypeptide consisting of a mature HKNG1 gene product having the amino acid sequence of SEQ ID NO:51.

15. An isolated polypeptide comprising an amino acid sequence encoded by the isolated nucleic acid molecule of Claim 6 or 7.

16. An antibody which selectively binds to the HKNG1 gene product of any one of Claims 13 or 14.

17. A method for treating a HKNG1-mediated disorder in an individual comprising administering to the individual a compound which modulates the expression of an HKNG1 gene in the individual.

18. The method of Claim 17, wherein the compound inhibits or potentiates the expression of an HKNG1 gene in the individual.

19. The method of Claim 17, wherein the compound is a small molecule.

20. The method of Claim 17, wherein the HKNG1-mediated disorder is a neuropsychiatric disorder.

21. The method of Claim 17, wherein the neuropsychiatric disorder is bipolar affective disorder or schizophrenia.

22. The method of Claim 17, wherein the HKNG1 gene encodes a HKNG1 gene product comprising:
(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:4;
(c) the amino acid sequence of SEQ ID NO:39;
(d) the amino acid sequence of SEQ ID NO:41;
(e) the amino acid sequence of SEQ ID NO:43;
(f) the amino acid sequence of SEQ ID NO:45;
(g) the amino acid sequence of SEQ ID NO:49;
(h) the amino acid sequence of SEQ ID NO:51;
(i) the amino acid sequence of SEQ ID NO:64; or
(j) the amino acid sequence of SEQ ID NO:66.

23. The method of Claim 17, wherein the individual is a mammal.

24. The method of Claim 23, wherein the mammal is a human.

25. A method for treating a HKNG1-mediated disorder in an individual comprising administering to the individual a compound which modulates the expression or activity of a HKNG1 gene product in the individual.

26. The method of Claim 25, wherein the compound inhibits or potentiates the expression or activity of a HKNG1 gene product in the individual.

27. The method of Claim 25, wherein the compound is a small molecule.

28. The method of Claim 25, wherein the HKNG1-mediated disorder is a neuropsychiatric disorder.

29. The method of Claim 28, wherein the neuropsychiatric disorder is bipolar affective disorder or schizophrenia.

30. The method of Claim 25, wherein the HKNG1 gene product comprises:

(a) the amino acid sequence of SEQ ID NO:2;
(b) the amino acid sequence of SEQ ID NO:4;
(c) the amino acid sequence of SEQ ID NO:39;
(d) the amino acid sequence of SEQ ID NO:41;
(e) the amino acid sequence of SEQ ID NO:43;
(f) the amino acid sequence of SEQ ID NO:45;
(g) the amino acid sequence of SEQ ID NO:49;
(h) the amino acid sequence of SEQ ID NO:51;
(i) the amino acid sequence of SEQ ID NO:64; or
(j) the amino acid sequence of SEQ ID NO:66.

31. The method of Claim 25, wherein the individual is a mammal.

32. The method of Claim 31, wherein the mammal is a human.

33. A method for identifying a compound which modulates expression of an HKNG1 gene comprising:
(a) contacting a test compound to a cell that expresses an HKNG1 gene;
(b) measuring a level of HKNG1 gene expression in the cell;
(c) comparing the level of HKNG1 gene expression in the cell in the presence of the test compound to a level of HKNG1 gene expression in the cell in the absence of the test compound,

wherein if the level of HKNG1 gene expression in the cell in the presence of the test compound differs from the level of expression of the HKNG1 gene in the cell in the absence of the test compound, a compound that modulates expression of an HKNG1 gene is identified.

34. The method of Claim 33, wherein the HKNG1 gene encodes an HKNG1 gene product comprising:
(a) the amino acid sequence of SEQ ID NO:2;
(b) the amino acid sequence of SEQ ID NO:4;

(c) the amino acid sequence of SEQ ID NO:39;
(d) the amino acid sequence of SEQ ID NO:41;
(e) the amino acid sequence of SEQ ID NO:43;
(f) the amino acid sequence of SEQ ID NO:45;
(g) the amino acid sequence of SEQ ID NO:49;
(h) the amino acid sequence of SEQ ID NO:51;
(i) the amino acid sequence of SEQ ID NO:64; or
(j) the amino acid sequence of SEQ ID NO:66.

35. The method of Claim 34, wherein the HKNG1 gene comprises:

(a) the nucleotide sequence of SEQ ID NO:1;
(a) the nucleotide sequence of SEQ ID NO:3;
(a) the nucleotide sequence of SEQ ID NO:5;
(a) the nucleotide sequence of SEQ ID NO:6;
(a) the nucleotide sequence of SEQ ID NO:34;
(a) the nucleotide sequence of SEQ ID NO:35;
(a) the nucleotide sequence of SEQ ID NO:38;
(a) the nucleotide sequence of SEQ ID NO:40;
(a) the nucleotide sequence of SEQ ID NO:42;
(a) the nucleotide sequence of SEQ ID NO:44;
(a) the nucleotide sequence of SEQ ID NO:46;
(a) the nucleotide sequence of SEQ ID NO:47;
(a) the nucleotide sequence of SEQ ID NO:48; or
(a) the nucleotide sequence of SEQ ID NO:65.

36. A method for identifying a compound which modulates expression or activity of an HKNG1 gene product comprising:

(a) contacting a test compound to a cell that expresses an HKNG1 gene product;
(b) measuring a level of HKNG1 gene product expression or activity in the cell;
(c) comparing the level of HKNG1 gene product expression or activity in the cell in the presence of the test compound to a level of HKNG1 gene product expression or activity in the cell in the absence of the test compound,

wherein if the level of HKNG1 gene product expression or activity in the cell in the presence of the test compound differs from the level of HKNG1 gene product expression or activity in the cell in the absence of the test compound, a compound that modulates expression or activity of an HKNG1 gene product is identified.

37. The method of Claim 36, wherein the HKNG1 gene product comprises:

(a) the amino acid sequence of SEQ ID NO:2;
(b) the amino acid sequence of SEQ ID NO:4;
(c) the amino acid sequence of SEQ ID NO:39;
(d) the amino acid sequence of SEQ ID NO:41;
(e) the amino acid sequence of SEQ ID NO:43;
(f) the amino acid sequence of SEQ ID NO:45;
(g) the amino acid sequence of SEQ ID NO:49;
(h) the amino acid sequence of SEQ ID NO:51; or
(i) the amino acid sequence of SEQ ID NO:64.

38. A method for identifying an individual having or at risk of developing a HKNG1-mediated disorder comprising the step of detecting the presence or absence of a polymorphism that correlates with an HKNG1 allele associated with the disorder, wherein presence of the polymorphism indicates that the individual has or is at risk of developing the HKNG1-mediated disorder.

39. The method of Claim 38, wherein the mutation results in production of a protein comprising an amino acid sequence that is different from the amino acid sequence of SEQ ID NO:2 or 4.

40. The method of Claim 39, wherein the mutation results in the substitution of a lysine for a glutamic acid at amino acid residue 202 of SEQ ID NO:2.

41. The method of Claim 39, wherein the mutation results in the substitution of a lysine for a glutamic acid at amino acid residue 184 of SEQ ID NO:4.

42. The method of Claim 36, wherein the method comprises the step of analyzing the sequence of the coding region of the human *HKNG1* gene by preparing and sequencing cDNA comprising a sequence that hybridizes under stringent conditions to the complement of a nucleotide sequence which encodes the polypeptide sequence depicted in SEQ ID NO:2.

TGCGTCACCTGCAGGCCCCGGGCCGCGGGGTTGGTTTCCACCCTGGAGGTTGCTGACACCCTGTGCCCTCGGCTGACTTC

CAGCCGGTGGCACAGACGCCTCCAGGGGGCAGCACTCAAGCGCATCTTAGGAATGACAGAGTTGCGTCCCTCTCTGTTG

CCAGGCTGGAGTTCAGTGGCATGTTCTTAGCTCACTGAAGCCTCAAATTCCTGGGTTCAAGTGACCCTCCCACCTCAGC

M K I K A E K N 8
CCCATGAGGACCTGGGACTACAGGACACAGCTAAATCCCTGACAGGG ATG AAA ATT AAA GCA GAG AAA AAC 24

E G P S R S W W Q L H W G D I A N N S G 28
GAA GGT CCT TCC AGA AGC TGG TGG CAA CTT CAC TGG GGA GAT ATT GCA AAT AAC AGC GGG 84

N M K P P L L V F I V C L L W L K D S H 48
AAC ATG AAG CCG CCA CTC TTG GTG TTT ATT GTG TGT CTG CTG TGG TTG AAA GAC AGT CAC 144

C A P T W K D K T A I S E N L K S F S E 68
TGC GCA CCC ACT TGG AAG GAC AAA ACT GCT ATC AGT GAA AAC CTG AAG AGT TTT TCT GAG 204

V G E I D A D E E V K K A L T G I K Q M 88
GTG GGG GAG ATA GAT GCA GAT GAA GAG GTG AAG AAG GCT TTG ACT GGT ATT AAG CAA ATG 264

K I M M E R K E K E H T N L M S T L K K 108
AAA ATC ATG ATG GAA AGA AAA GAG AAG GAA CAC ACC AAT CTA ATG AGC ACC CTG AAG AAA 324

C R E E K Q E A L K L L N E V Q E H L E 128
TGC AGA GAA GAA AAG CAG GAG GCC CTG AAA CTT CTG AAT GAA GTT CAA GAA CAT CTG GAG 384

E E E R L C R E S L A D S W G E C R S C 148
GAA GAA GAA AGG CTA TGC CGG GAG TCT TTG GCA GAT TCC TGG GGT GAA TGC AGG TCT TGC 444

L E N N C M R I Y T T C Q P S W S S V K 168
CTG GAA AAT AAC TGC ATG AGA ATT TAT ACA ACC TGC CAA CCT AGC TGG TCC TCT GTG AAA 504

N K I E R F F R K I Y Q F L F P F H E D 188
AAT AAG ATT GAA CGG TTT TTC AGG AAG ATA TAT CAA TTT CTA TTT CCT TTC CAT GAA GAT 564

N E K D L P I S E K L I E E D A Q L T Q 208
AAT GAA AAA GAT CTC CCC ATC AGT GAA AAG CTC ATT GAG GAA GAT GCA CAA TTG ACC CAA 624

M E D V F S Q L T V D V N S L F N R S F 228
ATG GAG GAT GTG TTC AGC CAG TTG ACT GTG GAT GTG AAT TCT CTC TTT AAC AGG AGT TTT 684

N V F R Q M Q Q E F D Q T F Q S H F I S 248
AAC GTC TTC AGA CAG ATG CAG CAA GAG TTT GAC CAG ACT TTT CAA TCA CAT TTC ATA TCA 744

D T D L T E P Y F F P A F S K E P M T K 268
GAT ACA GAC CTA ACT GAG CCT TAC TTT TTT CCA GCT TTC TCT AAA GAG CCG ATG ACA AAA 804

A D L E Q C W D I P N F F Q L F C N F S 288
GCA GAT CTT GAG CAA TGT TGG GAC ATT CCC AAC TTC TTC CAG CTG TTT TGT AAT TTC AGT 864

V S I Y E S V S E T I T K M L K A I E D 308
GTC TCT ATT TAT GAA AGT GTC AGT GAA ACA ATT ACT AAG ATG CTG AAG GCA ATA GAA GAT 924

L P K Q D K A P D H G G L I S K M L P G 328

FIG. 1A

TTA CCA AAA CAA GAC AAA GCT CCT GAC CAC GGA GGC CTG ATT TCA AAG ATG TTA CCT GGG 984

Q D R G L C G E L D Q N L S R C F K F H 348
CAG GAC AGA GGA CTG TGT GGG GAA CTT GAC CAG AAT TTG TCA AGA TGT TTC AAA TTT CAT 1044

E K C Q K C Q A H L S E D C P D V P A L 368
GAA AAA TGC CAA AAA TGT CAG GCT CAC CTA TCT GAA GAC TGT CCT GAT GTA CCT GCT CTG 1104

H T E L D E A I R L V N V S N Q Q Y G Q 388
CAC ACA GAA TTA GAC GAG GCG ATC AGG TTG GTC AAT GTA TCC AAT CAG CAG TAT GGC CAG 1164

I L Q M T R K H L E D T A Y L V E K M R 408
ATT CTC CAG ATG ACC CGG AAG CAC TTG GAG GAC ACC GCC TAT CTG GTG GAG AAG ATG AGA 1224

G Q F G W V S E L A N Q A P E T E I I F 428
GGG CAA TTT GGC TGG GTG TCT GAA CTG GCA AAC CAG GCC CCA GAA ACA GAG ATC ATC TTT 1284

N S I Q V V P R I H E G N I S K Q D E T 448
AAT TCA ATA CAG GTA GTT CCA AGG ATT CAT GAA GGA AAT ATT TCC AAA CAA GAT GAA ACA 1344

M M T D L S I L P S S N F T L K I P L E 468
ATG ATG ACA GAC TTA AGC ATT CTG CCT TCC TCT AAT TTC ACA CTC AAG ATC CCT CTT GAA 1404

E S A E S S N F I G Y V V A K A L Q H F 488
GAA AGT GCT GAG AGT TCT AAC TTC ATT GGC TAC GTA GTG GCA AAA GCT CTA CAG CAT TTT 1464

K E H F K T W * 496
AAG GAA CAT TTT AAA ACC TGG TAA 1488

GAAGATCTAATGCATCCTATATCCAGTAAGTAGAATTATCTCTTCATCTGGGACCTGGAAATCCTGAAATAAAAAAGGA

TAATGCAATAAACACAGTTGCAGGAAAGTATGTTAGCTATATACTATGAAGTACTCTTAGTTTACTTATGTTGAATGGC

TTAGCTATTAATACTCAAATTGAGTTAAAATGAAAATTCCTCCTTAAAAAATCAAACGTAATATGTATTACATTTCATG

GTACATTAGTAGTTCTTTGTATATTGAATAAATACTAAATCACCTA

FIG. 1B

TGCGTCACCTGCAGGCCCGGGCCCGGGGGTTGGTTTCCACCCTGGAGGTTGCTGACACCCTGTGCCCTCGGCTGACTTC

CAGCCCGGTGGCACAGACGCCTCCAGGGGGCAGCACTCAAGCGCATCTTAGGAATGACAGAGTTGCGTCCCTCTCGGTTG

CCAGGCTGGAGTTCAGTGGCATGTTCATAGCTCACTGAAGCCTCAAATTCCTGGGTTCAAGTGACCCTCCTACCTCAGC

M R T W D Y S N S G N M K P P L L V F 19
CCC ATG AGG ACC TGG GAC TAC AGT AAC AGC GGG AAC ATG AAG CCG CCA CTC TTG GTG TTT 57

I V C L L W L K D S H C A P T N K D K T 39
ATT GTG TGT CTG CTG TGG TTG AAA GAC AGT CAC TGC GCA CCC ACT AAC AAG GAC AAA ACT 117

A I S E N L K S F S E V G E I D A D E E 59
GCT ATC AGT GAA AAC CTG AAG AGT TTT TCT GAG GTG GGG GAG ATA GAT GCA GAT GAA GAG 177

V K K A L T G I K Q M K I M M E R K E K 79
GTG AAG AAG GCT TTG ACT GGT ATT AAG CAA ATG AAA ATC ATG ATG GAA AGA AAA GAG AAG 237

E H T N L M S T L K K C R E E K Q E A L 99
GAA CAC ACC AAT CTA ATG AGC ACC CTG AAG AAA TGC AGA GAA GAA AAG CAG GAG GCC CTG 297

K L L N E V Q E H L E E E E R L C R E S 119
AAA CTT CTG AAT GAA GTT CAA GAA CAT CTG GAG GAA GAA GAA AGG CTA TGC CGG GAG TCT 357

L A D S W G E C R S C L E N N C M R I Y 139
TTG GCA GAT TCC TGG GGT GAA TGC AGG TCT TGC CTG GAA AAT AAC TGC ATG AGA ATT TAT 417

T T C Q P S W S S V K N K I E R F F R K 159
ACA ACC TGC CAA CCT AGC TGG TCC TCT GTG AAA AAT AAG ATT GAA CGG TTT TTC AGG AAG 477

I Y Q F L F P F H E D N E K D L P I S E 179
ATA TAT CAA TTT CTA TTT CCT TTC CAT GAA GAT AAT GAA AAA GAT CTC CCC ATC AGT GAA 537

K L I E E D A Q L T Q M E D V F S Q L T 199
AAG CTC ATT GAG GAA GAT GCA CAA TTG ACC CAA ATG GAG GAT GTG TTC AGC CAG TTG ACT 597

V D V N S L F N R S F N V F R Q M Q Q E 219
GTG GAT GTG AAT TCT CTC TTT AAC AGG AGT TTT AAC GTC TTC AGA CAG ATG CAG CAA GAG 657

F D Q T F Q S H F I S D T D L T E P Y F 239
TTT GAC CAG ACT TTT CAA TCA CAT TTC ATA TCA GAT ACA GAC CTA ACT GAG CCT TAC TTT 717

F P A F S K E P M T K A D L E Q C W D I 259
TTT CCA GCT TTC TCT AAA GAG CCG ATG ACA AAA GCA GAT CTT GAG CAA TGT TGG GAC ATT 777

P N F F Q L F C N F S V S I Y E S V S E 279
CCC AAC TTC TTC CAG CTG TTT TGT AAT TTC AGT GTC TCT ATT TAT GAA AGT GTC AGT GAA 837

T I T K M L K A I E D L P K Q D K A P D 299
ACA ATT ACT AAG ATG CTG AAG GCA ATA GAA GAT TTA CCA AAA CAA GAC AAA GCT CCT GAC 897

H G G L I S K M L P G Q D R G L C G E L 319
CAC GGA GGC CTG ATT TCA AAG ATG TTA CCT GGG CAG GAC AGA GGA CTG TGT GGG GAA CTT 957

D Q N L S R C F K F H E K C Q K C Q A H 339

FIG. 2A

GAC CAG AAT TTG TCA AGA TGT TTC AAA TTT CAT GAA AAA TGC CAA AAA TGT CAG GCT CAC 1017

L S E D C P D V P A L H T E L D E A I R 359
CTA TCT GAA GAC TGT CCT GAT GTA CCT GCT CTG CAC ACA GAA TTA GAC GAG GCG ATC AGG 1077

L V N V S N Q Q Y G Q I L Q M T R K H L 379
TTG GTC AAT GTA TCC AAT CAG CAG TAT GGC CAG ATT CTC CAG ATG ACC CGG AAG CAC TTG 1137

E D T A Y L V E K M R G Q F G W V S E L 399
GAG GAC ACC GCC TAT CTG GTG GAG AAG ATG AGA GGG CAA TTT GGC TGG GTG TCT GAA CTG 1197

A N Q A P E T E I I F N S I Q V V P R I 419
GCA AAC CAG GCC CCA GAA ACA GAG ATC ATC TTT AAT TCA ATA CAG GTA GTT CCA AGG ATT 1257

H E G N I S K Q D E T M M T D L S I L P 439
CAT GAA GGA AAT ATT TCC AAA CAA GAT GAA ACA ATG ATG ACA GAC TTA AGC ATT CTG CCT 1317

S S N F T L K I P L E E S A E S S N F I 459
TCC TCT AAT TTC ACA CTC AAG ATC CCT CTT GAA GAA AGT GCT GAG AGT TCT AAC TTC ATT 1377

G Y V V A K A L Q H F K E H F K T W * 478
GGC TAC GTA GTG GCA AAA GCT CTA CAG CAT TTT AAG GAA CAT TTT AAA ACC TGG TAA 1434

GAAGATCTAATGCATCCTATATCCAGTAAGTAGAATTATCTCTTCATCTGGGACCTGGAAATCCTGAAATAAAAAGGA

TAATGCAATAAACACAGTTGCAGGAAAGTATGTTAGCTATATACTATGAAGTACTCTTAGTTTACTTATGTTGAATGGC

TTAGCTATTAATACTCAAATTGAGTTAAAATGAAAATTCCTCCTTAAAAAATCAAACGTAATATGTATTACATTTCATG

GTACATTAGTAGTTCTTTGTATATTGAATAAATACTAAATCACCTA

FIG. 2B

ACATTTTAAGCTACTTATAGTCCTTGGAAATAGCAACAAATATCTTAGTTATTGGACTATTATAACCTTAGTCATCTTATTACTGCTTG
ATTATGAGACACTCTCCCTGCTAATCCTTAGAACATCTTGGTTCTTGGTACTTGACTTTTAGCCCCTCTGACATATAGTTGATGTCAGA
GTGTCTGGCATTTCAGTAGTGCTCTATTTTACAAATCCCAGTAAACTGCTCCACTGTGGCTTGTTTATGTGTTAATACTGCTTGTTTTC
TGTTATAAATTATTTTTTGCTTTGGAGTAAGATATCATCATTTTGCATAGCTACAAATCTGAAGTTAAAGAAAATTTTAAAAATGTAAT
TGTGGGAAAATAACAAATAGATCTGCTGAGATGGAGGCTTTGACTAATGTTTTAATAACAGGCAACAAAACAAAGAGGCAGGATATTTT
GGTCACAACTAAACCTAAATTAAATCCTCATACAAAGCCCCATTAAGATAAATGCTCAAATTCTGGGAACATTTCACTTGCTTTGCCAG
CAATTTTACCCTTCAGAGGGTGTGGATCTAATCAGGGGAACAAACTACCCTGGGCTTAATTCTCATTAACAGGGACTAATTTGTCAAAG
CGGCAGTACTAGCTGAAGTGATGGGTATGGAAGCATTCACTGTGAGGATTTTGCTGAGGTGCCTGGCACAGGGTAGGGGAACTCACCCA
GGCTGCAAGATGCTAACAGTTCAGGTTCAAGGTCTTAGTGTGGACTAAGGTGCAGTCAGGATGGGAACAGGTGCAACTTGGGCCAACAT
CAGTATGAAGGGCCTGATCTGAGGGCAGGGGAAGGAGGGGGCATTCTGGGAAGCAAGAGTTCCTGGTATCCTGTTGACCAGAGTCTTGG
CCCAAGGATCAACGTATGAATTAAAGTAGAAATACCAGAAACAAAGAAAGTTGGCAGAAACTAGGAGAAGCAGAGTCTCAGCCAACTGG
ACTGGGCTCAGCCTTGGCTACTGGCCCGGCAGATGATAGAAGAGAAAACCAGGAACCCAGGCTGAAGCCCAGTGGTTGGGCTGGCCACA
CACCATGCATAGCCTTAAAGGGGTGGCCTAAGGGCATGGTCCGCTCCAAAAAAGGAAAGGGGGCCCCAGAATATTTCTGAATCCCACTC
ACTGCCAGGGAAGAACCTCTCAATTCACTCAATAGTGCATTCTCCTGCTTCTCAATAGGCTAATACTCTAGAGAATATGGGGACAAGGG
GAGGAGGGTCTAGTGGAACAGGTCTAAACTGGCGTTTGAATTTTAAGATAAGTTAATCATACATTGGCTGGGTCAGCCATGTCTCTTAG
TCTTTACAAAAGTAGAACACAAAAAAATTCAATGGAAATCTACAGACACCTATTTGCAGATGAGGAAACACGGCTATGAAGATTGGGAA
GATTGGGAAGAACTGGCCAGGTGTGGTGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCTGGTGGATCACTTGAGGTCAGGA
GTTGGAGACCAGCCTGGGCAACATAGTAAAACCCTGTCTCTACTCAAATTACAAAAATCAGCAGGGCGTTGTGGTGCCCACCTGTAATC
CCAGCTATGCAGGAGGCTGAGGCAGGACAATCACTTGAACCTGGTAGGCGGAGGTTGCAGTGAGCCAAAATCACGCCACTGTACTCCAG
CCTGGGTGACAGAGCAAGACTTTGTTTAAAAAAAAAAAAAAAAAAGGGAAGAACTAAAAATGTAATTTTCAAGGGGCTATCACAAATGGT
CCCAATAAAGAGAAAGCAGGACTCATGTTTAAGAAACCCATGAGATGTGTATGGACCTCATGGAAGAGCTCTTGCTTTCTAATGATCTA
CGTAACAGATGAAAAGCAGAGCATAGGGCTAAGGATGAAAATACAACAGTAATAAGGTATTAATATATTATTAAGAAAGCTAATGCTCC
ACATAAGCAGAGGACATTAAAGGGACTTTTTTTTCTTAAGGATATCTTAATGTTTTAAATGAGAAGACATAGAAAGGGATAGGTCCAAC
TCTTGGGATTGTTGCAGGTTGGTTTCCATCGGAAGCACTCTGAGTCTGAGATTTGTATGCAGAAAATTAATTTGAATGTGCTTTTCAGA
TCACCCAGGTGGGGGAGGGAGGAAACCAGGACTGGGCAGAGAGAGGCTGGGCTGTAACCAAGTCACAACAAAGGTGTCAGCTGGTCCCA
TGGTGAATTCTGGACCTAGGATGGCTGATCCCAAGGCATTCCAAACTGGGGCAAGGAAGTTGTGCTTTAAAACTTCTCATTGACTGTCA
GTCACTGGGCATGAGCAGTCCCCAGGAAGGGGGGATGACCTTGAGCAAGGTGGATGTCTTCAGCCAAGGGCAAYCACTGGGAAGGAGAA
CCCAGCTATGAACTGTCAGCTGCCAACACTCCCAGCATCTGAGAGGATGAGGGCTTCAATTCTAAGGGCAGGGGCTCCAAGGGCAGGGG
TACGGATGGTGGAATCTGGGCAGTACCTTGTGGCTTCCACTACAGTCCACCCCTTGCACCACTTAGTTCCACTGGCTTTTTTTTTTTTT
TTCTTTTCTGAGACAGTCTCACTCTGTCACCCAGGCTGGAGTGCGGTGGCACGATCTCGGCTCGCTGCAACCTCCGCCTCCCAGGTTCA
AGCAATTCTTGAACCTCCTGAGTAGCTGGGACTACAGATGTGTGCCACCACACCCAGCTAATTTTTTGTATTTTTAGTAGAGACGGGGT
TTTACCGTGTTAGCCAGATTGGTCTCGATCTCCTGACCTCATGATCCGCCTGCTTTGGCCTCCCAAAGTGCTGGGATTACAGGTGTGAG
CCACCGCACACAGCCAGATCCACTGGCTTCTATATAATTTCTGGGTGAAGCTAATTCAGGATTCTGATGGACCTGTCTTCCCGAGGGAA
ACTTGTAAAAGGAAAGTTAGAGGGACAAACTATAGCCCCTGCCACAGCAGCTGCTGTCGAGGACAAAAATGGTGCTCCTCATTTCCCTT
AACCACCTGACCTAGATTCCCCTAACCCTTAGTGGGCACCTCTGTGGATGGAAGTGGTGGCTCACYKGKKGGRWKRWYCMRRWYYCWYM
YCCCTGAGTGGTCTGAGCTCCCAGTTACCAGGCCCTTCTCAGGCTGTGGCTGTTGCACTTACCTCCCCAGCCATCCCCCACTTTTTTTT
CTTGAGACTGGGTCTTGCTCTGTCACCCAGGCTGAAATGCAGTGGCATAACCTCAGCTCACTGCAGCCTTGATCTCCCAAGCTCAAGCC
ATCTTCTCACCTCTGCCTCCCAAGTGGCTGGGACTACAGGCACATGCCACCATGCCCAGCTAATATTTTTTATTTTTTATTTTTTTGTA
GCAATGGGATTTTGCCATGTTTCCCAGGCTGGGCTTGAACTCCTAAGCTCAAGCTATCCTCCCACCTCTGCTTCCCAAAGTGCTGGGAT
TACAGGCTTGAGTCACTGCATCTGGCCACATTTATTCCTTTTAAACGTTAAAATTGAATGCAGGATCACTGAGAGACAGGTGAGTGATT
ACCAGGGTGCCAAACATACCCTTCTCCTCCTTTCCTGCAGCTCTACCTCCTCCTGATGATCAGGACAATCATGTATGATGACTCCTTTC
CTTGACTGCTGCTCTCTCAGAAGGAACCCATTGTGTTGGGTGAGAACACATCATTTGAAATTTAGTAAGACTCTTGCTGTGCCTATGGT
AGAAGCATTCCCTCTCTGGGGCCAAGATCTTTAAATGCACAGAGTCCAAAGTCGTGGGAACCAAAGCAGAAATTAAAAAGGAGATGACT
GGGATTATGGTAAGAACTGTTTCCACCCTTGATTTGCTGCACCCATGTGTTCTACCTAGGAGATAGCACACCATATACTGGTTATTCAT
TTGGATTACATGCTGCATCCCGGAGAATGGGCACTGCATTCTCACTGGTCATCATGTCAGAGCCTGCGCTGCAGAGGCTTTCCCATTGC
TCTGTCAGTGTGTTATAGGGTCAGTGGATTTCATGGTCATGTGCCCACTGCTGCACCTCCATTCTTGTAAAATGGGTCCTCTGGTTCAA
TGTGATGCCATGTGGGATCTTGTGTCAATAGAATAAATACTCAGATGTTCTGGCTGAAGCTTTACAAGCAGAAAAGGCCAACCGATGAC

FIG. 3A

TGAAATAAGCGTTGAGCCCAGTCAAGATGAGTTCCTGCTCTTTCCAGGATAGACGGAGTCTAGTGTAGATCACTTGACATCAAGAGACT
GGCTGGTCTCCTTGAGGGATGGTGCTGTTCTGCATTCATCATCCTTGATGAATGAGGGACCCTGCTATTGGGCTCATGTACAGCCCCCA
TCTCTGCCACAATGAGCGCTCCATTCATGTTCCTATTGTGCCAACACTAGGGTGTCTGTAATCACTGAAAACATTATTGCTATCATTAT
TATTATTTTTTTTTTTTGAGACAGAGTCTCGCTCTGTCGCCAAGGCTGGAGTGCAGTGGCACGATCTCAGCTCACTGCAACCTCTGCCT
CCCGGCTTCAAGTGATTCTCCCGCCTCAGCCTCCAGAGTAGCTGGGATTATAGGCATGCGCCACCACGCCTGGCTAATTTTTGTATTTT
TAGTAGAGACAGTCTTTTGCCATATTAGTCTGTCTGGTCTCGAACTCCTGACCTCAGGTGATCTGCCCGCCTTGGCCTTCCGGAGTGCT
AGGATTATAGGCGTGAGCCACCACTTGCTATTATTATGTTGAGAAAACTGTTTTCAATTATAAATAAGAAAAAATAAAAGATTATATTT
TGCCTTTATTCCTTCTCTAATGCTGTTCTTTAAGTAGATGTGAATTTCTGAACTACATACTTTTTCTTTACTCTTGAGAGGTTGTTTGG
AGGTTCCAGCAGGGGACCACAGCTACTCGTATACCCTTGACCAAAGACTGGTCCTTGTCTATCAAGGATGGTCGTCTTCTTCCACCAAG
CACACAGCTTCTGGAGGGACGCACATGGAGTGGTGAGGGAGGAAGGGGACACCCGCCTAGCCAGCTAGATCAGCCAAGCAGAATAAACC
CTGGTAGTCAATGGGGTGACAGTGTCGCAGCCAGATTGCCCTCACATCCAACTCTTAGTGATCTTCTCTTAACATTTCTTGCAAGGCAG
GTCTACTGGTACAAATTCTCTAATTTTTGCTTGTTTGAGAAAGTCTTTGTTTCTTCTTCACCTTTTTTTTTTTTTTTTTGGAGACAGAG
TCTCCCTCTGTTGTCCAGGCTGGAGTGCAGTGGCCTGATCTTGGCTCACTGCAAACTCTGCCTCCCAGGTTCAAGTGATCCTCATTCCT
CAGCCATCTGAGTAGCTGTGGTTACAGGCGTGTGCCACCATGCCTAGCTAAATTTTGTATTTTTAGTAGAGACGAGGTTTTACCGTGTT
GGCCAGGATGGTCTTCAGCCTTCTTAACTTTTAAAGGATAATTTCACGGGGAGAATTCTAGGTTAGTGTATTTYTCTTTCAATACTTTA
AATATTTCACTCCACTTTCTTCTTGCTTGTGTGGTTCTGAAGATAATGATATAATTCTTATTCTTGTTTCTCTGCAGGTAAGGTGGTTT
CATACCTCTGGCTTCTTTCGAGAATTTCTCTTTGTCTTTGATTTCCTACAGTTTGAATATGATATAATTATGTATAGACTTGGGGCTAT
TTATCCTTTCTGGTGTAGTCTGAGCTCCCTAAGTCTGTGGTATGGTGTCTTGTAATTGATTTGGGAAAATTCTCAGTCATTATTACTTC
AAATATTTCTTCTGTTCCTTTGTGTTTTTTTAACTTGTGCCAACTTTTTAATTGATACATAGTATTTTACATATTTATGGGGTACATGT
GATACTTCATTACCTGCATAGAATGTGTAAATGATCTAGTGAAGGTGTTTGGACTATTACCTTGAGTATGTATCGTTTCTATGTGTTGG
GAGCTTTTCAAGTCCTCTCTTGTAACAATTTTGAAATATACAATGCCTTGTTGTTAACTAGTCACCCTGCTCTGCTCTCAAACACTAGG
ATTTATTCCTTCTGTCTAACTGGGTGTTTGTACCCATTAACCAACCTGTCTTCATCCCCTCTACCCACATACCTTTCCCAGCCTTGGGT
ATCTATCATTCTACTCTTTACCTCCATGAGATCAGCCTTTTTAACTCCCACATATGAGTGAGAACATGTAGTACTTGTTTTGCCGTGTC
TGGCTTATTTCACTTAAGATAATGACCTTTTATTCCATCCAGGTCACTGCAAATAACAAGATTTCATTGCTTTTTCTTTTTATGGCCAA
ATAGTGTTCCATTGTTTATATAGACCACATTTTACTTTATCCATTTGTACATTGATGAACACTGAGGTTGATCCATATCTTGGCTATTG
TGAATAGTGCTGCAATAAACATGGGGGTGCAGGTATCCCTTTAATATACCGATTTCTTTTCCTTTGGATAAATACCCAGTAATGGGATT
GCTGGATCATGTGGTAGATGTATTTTAAGTTTTTTGAGAAACCTCCATACTCTTCCATCATGGCTGTATTAATTTACATTCCCATCAAT
AGTATATGAGTTCCCTTTTTTTTCTGCATCCTCACCAGCATCTATTATTTTTGTCTTTATAATAATGGCCTTTCTAACCAGGGTAAGAT
GATATCTCATTGTGGTTTTGATTTGCATCTCCCTGATGAGTAGTGATGTCAAGCGTTTTTCCATATGCCCATTGGCCATTTGTATGTCT
TCTTTTGATGAAGTCTGTTTGTGTCCTTTGCCCACTGTTTATGCTCCTTTTTTTCTTCTCTCTCTGGTATCCCCCTCACACATATATCA
GACCTTTTTTAATTGTCCCACAATTCTTGCATTTTCTGTTCTTTTTCATTCTTTCTTCTCTTTGTATTTCAGTTTTGGAAGTTTCTATT
GATATTCAAGCTCACTGATTCTTCCTCTGGCTCTGTTCAGTCTATTAATAAGCCCTTCAAAGCCTTTCTCTCTCTTTCTTTCTTTCTCT
CTCTCTCTTTCTCTCTTTCGTTCTTTCTTTCTCTATTTCCTTCCTTTCTTTCTTTCTCTTTCTTTCTTTCTTTCTCTTTCTTTCTTTCT
TTTTCTTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTTCTTTCTTTCTCCTTCCTTCCTTCCTTCCTTCCTTTCTTTCTTTC
TCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTT
TCTTTCTTTCTTTCCTTCTTTCTTTCGACCAGTTCTCACTATGTTGCTCAGGCTAGCCTAGAACCCCTGGGCTCAAGTTATCCTCTCAG
CTCAGCCTTTCAAGTAGGTGGGACAAATGCGCCATTCTATCATACCCAACAATTCCTCATTTCTGTTACAGTGGTTTTTATTTCTAGCA
TTTTCTTTTGATTCTTTCCTAGAGTTTCCATCTCTCTGCTTACATACACATTTGTTCTCTCATATTTTCCACTTTTTCCATTAGGGCCT
TCAGCATATTAATTAGTTATTTTCAATTCTAGCCTGATAATTCCAAAATCTCGGTTATATTTGAGTCTGTATCTATGCTTGGTTTGTCT
CCTCAGACTGCGTTTTTTCCTTTTAGGATGTCCCTTATCATTTTTTGTTGAAAACAAGACATGATGTATCAGATAAAAGTAATTGAGGT
AAACAGGCCTTTAATATGAGGTTTTATGTTTATCTGGCTTGGAGTTAGGCTGTGTTTACTCTTTGCTGTAACTTTGGTGCCAGAGGCTA
AAATTTCCTCTGGTGCCCTTGTTTTTGTCTCTCCTGTTATGTTTGTGTTTCCACAGAGTCTCCGTGAATATGGTGTGAGGCTTGAAGTT
CTTTAGCTGTAACCCCTCTTATTATACAGGAGCCTTACGGATGTGGTGGTAATGTGGGAGGGTGGGCTTAAGTATTCAGCAGTCCTGTG
ATCAGGCCTCAGTCTTTTAATAAGCCTGAGTACTTCCCTTTCCCTTTCTGCATGTTAGAGTGCCCTGGAGTTGGGGGTATCCATTACCC
CAGGTTGGTAGGCTTTGGTAAAACCACAGTCTATCAAGCTGTGGTAAAATAGTTTCCCTGCAGTCTGGCTTTGTTAAGGATAACAGAGG
GCTCTGGGGGTGTTTCAAAATTGCTACTTTTCCTCTCTCCCTGTCAGAAGCACAAGGAGATTTCTCTTGATCTTCACCCTGAGAGTCTG
GTGGGGTTCCTGGAGGTAAAACTCAGGAAAGTGTGAGGGCCTCCACACAAAGGGTCTGCTGAAGTTTGTTCCATAGCCTCAGTTCTCTA

FIG. 3B

ATGGATCTAAGAAGAGTTATTGATTTTCAATTTGTCCAACTTAATTCTTGTTTTGAAGACAGAAGTGATGACTTCCAAGCTCTTTATAT
GTTGAACCCAACCCCATATTATTTTCAATTAGCAATTGCATATAGCAATGGTACATTGCATTTATAGAAATATAATTGATGTTTGCCTG
TGTATCTTTTTTCCTATTATGTTGCTGAATTCATTTCTTAGTTCTAGGAATTTTTCAAATACATCCCTTAGGATATTCTGTATACATAA
TCATGTCATCTGCACATAGGGACAGTTTTATTTCTTTTTCTAGTCTGTATTTCTTATTTCCTTTTCTTGCCTTATTGCAGTGGCTAGAA
CTTGCAGCACTATATTAAAATAAGAGTGGTAAAAGTGAACATTCTTTCTTTGTTGCTGATCTTGGGGGAAAGTATTCAGTCTTTCACC
ATTGAGCATAATGTTAGCTGTAGGTGTTTTAAATCTTTATCCAGTTGACGAAGTTACCCTTTATTCCAATTTTTCTGAGAGTTTATATC
ATAAATGTGTTAAATTTTGTCAAATTTTTTTGCATGTATTGATATGATTATGTGGTTTTTCTTCTTTAGTTACTGCAGTGGGTTGCATT
GATTGATTTCTATTATTGAACCAGCCTGCATTCCTGGAATAAACCCCATTTGGTCATGATGTATAATTCTTTTTTTTATATTGCTGAAT
TCTATTTGCTAATATTTTGTTAAGGATTTTTGCATCTGTGTTCATGAGGGATCTGGGCTGGTAGGTTTTTTTCCCCCCTGCAATGTCTC
TGTCTGGTTTTGGTATTAAGGTAATTTTTTTTKTTTTKTTTTGAGATGGAGTCTCGCTCTGCTCACCCAGGCTGGAGTGCAGTGGCAC
GATCTTGGCTCACTGCAACCTCCACCTCCCAGGTTTAAGCGATTCTCCTGCCTCAGGCTCCTGAGTAGCTGGGACTACAGGTCACACCA
CCACGCCCGACTAATTTGGTATTAAGGTAATATTATCATCATAAAATGAACTGGGAAGTGTGCCCTCTTCTTGTATTTCTTTTTTTTTT
TTTGAGACAGTCTTGCTGTTGCCCAGGCTGGAGTACAGTGGTACGATCATGGCTCACTGCAGCCTCAAACTCCCAGGCTCAAGTGATCT
TCCTGCCTCAGCCTTCCCAGTACAGGGGCAGGCTACCACATCTGGCCAATTTTTAAATTTTTCTTTTGTAGAGAGGGGTCTCACTATGT
TGCCCAGAGGATCTCAAGCAATTCACCTACCTTGGCCCCTCTTCTTGTATTTTATGGAAGAATTATTGGTGTCAATTCTTCTTGAAAGT
TTCGTTAGAATTCTTCAGTGAAGCTGTATGGGCTTGAAGATTACTTTTTTTTCTTTTTTTTTTGAGATGGAATTTCACTCTTGTCGCCC
AGGCTGTAGTGCAGTGGTGTGACCTCTGCTCACTACAACCTCTGCCTCCCACGTTCAGGTGATTCCCCTGCCTTACTCAGCCTCTGGAG
GAGCTGGGATTACAGGCACCCGCCACCATGCCCGGCTAATTTTTTGTATTTTTAGTAGAGACGGGGTTTCACCATGTTGACCAGACTGG
TCTCGAACTCCTGACCTCAAGTGATCCACCCGCCTCGGCCTCTCAAAGTGCTGGGATTACAGGCATGAGCCACCGCGCCCAGCTGAAGA
TTTCTTTTTGGGGAGTTTTAAATTATACAATCAATTTGCTTAATAGGTATAAGCTATTCAAGTTATCTATTTTATACTGGATGAGTTGC
AATAGTTTGTGGTTTATGAGTTTATATGGTCCATTTCATCTGAGGTATAAAATTTAYTTGTGTAGTATTGTTGGTAGTATTCCCTTGTT
ATCTTTTTTATGTTCACATGGTATATGGTGACAGTCCTGGTTTAATTCCTAGTATTAGTAACTGGCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTGGTCAGTCTTTCCAGAGGTTTGTCAATTTTGTTGACTTTTTTCCCCCAAAGAATCAGCTCTTTGTTTCATGGATTTTCT
GCTTTTCTGTTTTCAACTTCATTGATTTCTGCTGTTTATTATTTCTCTCCTTCTGTTGGTTGTGAGTTTGTTTTGCTTTTCTTTTTCTA
CATATTCGATGTGAAATCTTACATTATTCACTCGGGACTTTTCTTCTTTTTTGATGTATGCATTTAGTATTCTAAATTTACTTCTKAGT
ACTGCATACTGCTTGAACTATGTCTGACAAATATTAATATATTGTTTTTAAATCTTTATTCAGTTCAGTGTATTTTTAAAATTTCCTTC
TCTGCCTCTTCTTTGATTTGTTATTTAGAATTGTGTTGTTATTTTCCGAGTATTTACATTTTCCTCTTATCTTTCTGCATTGATTCCAT
CGTAGTCAGAGTGCATGCTCTGTACAGTTTCAGTTCTTTCAAATTTATTGAGCTTTGTTTAATGGATCTGGATACAGTTTATCTTGGCA
TATATATATATATACACACACATATGTATGTGGGCGCTTGAAAAGAAAGCGTATCTGCTGTTTGGTGGAATGTTTGGAGTGTTCTATAA
GCGGTGATTAGATACTGTTGGTTGATGATGTCATTGAGGGTCCGATAACCCTACTGATTTAAATTTATTTAGTCTGTCAATTATTCAGA
GAGAGAGGTGTTGAACTCTGCAATGTGAATTGTGGATTTGTCAATTTCTCCTTTCAGTTCTATTAGTTTTTTCTTCACATATTTTACAA
CTCTGTTGTTTGGTGCATACACATTTATGCACCAAATTTAGGATTGCTATAACTTCTTGGTGGATTGACCCTTTTACATTATATAATGT
CTTTTTCTGTCCCTGGTAATTGTGGTTGCTCTGAAGTCTATGTTATCTCAATATAAATAGACAACTCTGCTTTCTTTTGATTAATGTTT
ACATGATACATCTTTTTCTATTCTTTTACTTTCAACTTACTTATATTATTATGTTTGAAGTGAGCTTCTTGTAGACAGCATGTAGTAGG
TCATATATGTACATAGATATATATATATTTTTTTGAGATGGTGTACTCTGTCACCCAGGCTGGAGTACAGTAGTGCTCACTGCAACCTCTG
CCTCCTGGGTCAAGTGATCTCGTGCCKCAGCCKCCCCAGTAGCTGGGATTACAGGCACGCACCACCATGCCCAGCTAATTTTTGTATTT
TTAGTAGAGACGGGTTTAACCATGATGGACAGGCTGGTCTCGAACTCCCGACCTCCAGCGATTAGCCCACCTTGGCCTCCCAAAGTGCT
GGCATTACAGGTGTGAGCCACCGTGCCTGGTTTAATATTTTTAATCCACTCAGTCTTTGTCTTCTACTGGTGTACATAGACATTCGCAT
GTAATGTAAATGTTGATATGTAAGAGCTTGAATCTGTTATGTTTTTGCTTTCTCTATGTTTTCTCAATTTTTAATTTCTCTGTTTTCTT
TTTTTCTGCTTCATATTGGCTAATGAACACTTTGAATCATTCCATTTTGATTTACCTATAGTGTTTTTTAGTGTGTCTCTTTGCATAGC
TTTTTTAGGGGTTACTTTAAGTATTTCATTATATGTACATAACTTATCACAGTATATTGGTATCGTTATTTTACCAGTTCAAGTAAAGT
ATGGAAATGTTTCCTCTCTACATTCCTTTACCTCATTTATAATATAATTGTCTTAGGTATTTCTTGTACATACATTTTAAACCGGATGA
GTGTTATTTTTGATTTAGCTATCAAATAATTCCAAAAACTCAAGAAAAAAAGGAAAGCTTACTATATTGACCCATATTTTCATTCACCA
TGTTGTTTCTTCCCTCTTTATGCCCCATAGTTCCTTCTTCTATTGTTTTCGTTTAGAGAACTTCCTAGCCATTCTATTGGGGTAGATCT
CCTAGTGACAAATTCTCTTAGCTTTCTTTTCTCTGTGAATGTCTTTATTTCCCTCTTTGTTCCTGGAGGACATTCTCACTGGATATAGG
ATTCTTGGCTATTGGGTCTTTTCTTTTGGCACTTTTGTAAGTGTGCAGCCTGCTGTCAAAATAAAAATTAAAATAAAATAAAAATGAAT
GTTTTCCTTTGCTACGTTCATGAAAGTATAATTCACTGAATGAGGAGGGACACCCATCTCTATAATCTGGAGGCCCATGCTCACCTCTG

FIG. 3C

AATAGTACATTTGCAGAGAAATTGGGGAAATCAAAGTCTGTTGAGACCAGCAAGATAAATAAGGCAAAAGGATACAAAACCATATCCAA
AGAGAAATGGTTTAAAGGAACTAAGGCTGTTTCTCCTAAAAAGAAAATAGTTGGAGACATGTGACCTCCAAAGAAACAGGACTTTTTCT
ATGGGGCTCCAAGGGGTTTCTATGAGAGAATGATAAAGGAGAGATTTCAGCTTAGTCTCAGGAAGACTTTTCAACAACCAAACCTGCCC
AAAGATGGACTGCCCTGCCTAAGGATTGTGTTCTGACATTAAGGGTATGGAGGTATGGGTTAGATGAATATTTTACCAAAATGCCATAG
ATATTTCAGGCTATTGATGTTGTAATATCATACTAGGCAACTCCACTTCAATATGAGTCTCTATGATGTAAAATGAAATAGGATGTGTT
TCGATAGAGAGTTGCAGATTTCATTTTGATGTTAGCGACCACACAAAATTACTTTCCCTACATAAGAACATGTTATTACTCTAGTTGAT
GATGACTGCTTATGGGAAATGTGTCTGCTTTGTTAGGAATCTTGCCTAATATATGTATAATTCAAGATGGTATTATAAAGTGACATATA
TGATTTTAACATTTGCACTTAAAATAACACTTATTCTGTACCATGMASTGTCTAGGAGCTTCTACATATTCCATTATTATCTTTATTTT
ACAAGACAGGGAACTAAGGCATGGAGAGATTGAGTAATTTGTGCAATATTACCTACCTAGTAAGTGGTAAAGGAAAGATTGGAACCCAT
TCTGGCTCCAGGATCCAGGCTCAAAGCCAATATACTATCCACCACCCCAACTCTTTAGTTTGATCAATTTGTCAAATTATTTTACAGTT
ATTTATCTGTAAATTAAGGGGATAATTGCCCAGTCAATAAATGTGTCCCCTTCAAAGGTTACATACTTAACCAATGGTGCTACTGGGCT
CAGAACATTTTTGGAACTACGATTTTGGTGGCAACCAAAAAACCTCCAGTACATTCCTCTGAACATTCTCCAGAGGCAAGTCTTTCTCC
ATGGAGACTGGGCTTCATTTTTTGAATTAGCCTGAAGTTGTTTGAGGTCAAATCTGATGAAAAGAGCGGCTGGGGAAGCTGGATATTTT
CGTTCGTGATTTAAAACAGTAAATGCCACCTAAATGAGAAGGCTACTTTCTTTGAATGTTTTGTAAACTGGCTTTGAAGGTACTTCTTT
AAAAAAGAAGCACAAGAAAGACGGTGACTGGCAACAGCCTCACTGGAATACGTCTCTAATCATCAAGGCAACCCACACTCATTTGGATG
TGTGCATCCGGTGATGTTATTATTTTTAAAGTTATGTGCCACAAAGATGCATTCTTTGCTATACAAAAGAGCTGTTGTTAAATTTATAA
AGATATAAAAAGGGGAAAGGAGAAGGCACCAAATGGAAGATTCTTAGGCATTAAGTGCTCAGACAGCATAGATCTTCATTAGATGACGT
CAGGGAGAAGAGACACAGACTTTGCCATCTCAGGTAGAAGTATCAAAGTCATCAGCCTCCTAGTAAGACAGACCTGGGTTTGAAGCTCT
GCACAGCCATTTCCTAGCTGGTCTGGGGAAAAATTACTTCTTGAAGCCTCAGTGTCTTTATTTGTAAAGTAAGTGGAATTATATTACCT
TGTCAGGATGTTGTCAGAATTAGAAATAATTTAAAGAGGTCCAGCACGAGCAGGTCAATCAAGGGAAGATGTTAAAAATAACAACAGGT
GAAATGTACTCCCAAAAGATAAAGTGGATACATAGATGAATCTTCCTCACACACAGAGTATAATAACCTCAGAAAAAATATTGCCTAGAG
TAAACATGCCTCCCAAGCCAACGTTCATCATCCAGGAATACGGAGAGGATGTTTGGGATATGGGGGGCATGAAATTTTTACAATTGTAGG
GCCCTTTAACAAGGGTAGACTTGCAAGTTGCACTGMCTTTCCTGCCCTCCTCTGGCTACCTGTTCCAGCATCCAGAGTTTGTGAACCTG
GGGMCCAAGGACAGCACCCTGGCATGGGCAGGCCCACTNGGCGACTCTCTCAGGGCTGCTGCAGCTGTGTCAGTGTCCCCACAGGGAGN
CTGACATCCAGCCATGACCATCGCATTAAGCCCAGCAGTCAGGGCAGGGGAGCAACTGCTCAGAGGCACCTTTGACCCACTACTTTTTT
CCCCTCCTGCTTTATCTGCCCAGAGCGAGGCTCTCTTTCTAATGTGTACAAGGCGTTCTACCTATGACTCGTGGTCCTGCCATAGAAAT
GCTTTTTTTTTTTTTAACTGAATTAAGTTGCCAAGTTTGAAAAATCAGAATTTCACATAAGATCCCTATTTCTGTCTTCTTTTGAAAAA
CTGAATGTTCTTTCCACAGTGAGCCCACATTCCTTCCTGACGACCATCACCGTTCAGCTGGAGTAGAGAGGGCTCTGCTGGCTTCAGAT
CCGGACGCGCAGGTCCTCTGCAGGCCCCGCCCACCCGGCGTCACCTGCAGGTCCCGCCCACCCGGCGTCTGCAGGCCCCGCCCACCCGG
CGTCACCTGCAGGCCCCGCCCACCCGGCGTCTGCAGGCCCCGCCCACCCGGCGTCACCTGCAGGCCCCGCCCACCGGCGTCTGCAGGC
CCCGCCCACCCGGCGTCACCTGCAGGCCCCGGCCCACCCGGCGTCTGCAGGCCCCGCCCACCCTGCGTCACCTGCAGGCCCGGGCCGGCGG
GGTTGGTTTCCACCMTGGAGGTTGCTCACACCCTGTGCCCTCGGCTCACTTCCAGCCGGTGGCACAGACGCCTCCAGGGGCAGCACTC
AAGCGCATCTTAGCAATGACAGGTGAGARCATCCTCCGGGCCCCAGATTTCTCTCCTCGCCGCTCTTGCCCATTTCTCCGGAGAGCCAG
AGAAAGCCGCTCCCAAGTCCAAGGCCGAGCTCCGCAGACGCCCGGCCCCTCCGGCGCGGACAGAACAAAGCCATTGTTCTTGCCGGGGA
AGGTAGAAATACTGTGGGCTGCTTCAGAGGCTGCCGAGCAAAACTCAGGCAATCTCCTGGGCTGTTCCAATACGTTTATTCTCTTTTTC
AAAACAGGAGGAGGAGGTAGAGGCGGGGAGACACACCATCCCTGCAAAACTACTGGCAAAAACTAAGCGGAGCCGGGTGTGGTGGCTCA
CGCCTGTAATCTCAACACTTTGGGAGGCCGAGGGGGGCCGATCACTTGAGGTCAGGAGTTGGAGGCCAGCCTGGCCGGCATGGTGAAAC
ACAAAAATTAGTCGATTGTGGTGGTGCATGCTTGTAATCCCATCTACTTGGGAGGCTGAGGCAGGAGAATCGCTTGAACCCGGGAGGCG
GAGGTTGCAGTGAGCCGAGATTGCGCCACTGCACTCCAGCCTGGACAACACAAGTGAGATTCTGTCTCAAAATAAATAAATAAATAAAC
CCAAGCAGAAAAAGAATCACTCTGAAAACGATCACATCTAACTATCAATGCTCATACAGTTTATGGAATTATCAGCCCAACTTGATAAA
ATCAGTATTTGAGGAAACTGTGGATAAGCCCCCTGATTTCAATCCCCATTGTGCCAGGTCCTGGTTAACTGAGGTTAACGAAGTAAAGA
GCTGCAGACACTATTAACTGCTACCTTAAACCGATTACTCTAGCTTAGCCTACTTTCCACGTACAGATTTTACCAGTGGACAACATGAT
GCTTTATCTTGTTTTTCTCTCCCTGGGACTTTTCTCCAGACATTGAAAACAGAAATACTAATAAGGCCACTTTTACCTGCCTGATGCAA
GAACAGAATTTTCAAACTCAACATTAATGCAACTCCTCAGTCCCTGACAATGGCGGGTGGAAAAGTTTCTAAAAATATGCAGCAGCACA
ATTATCGGGAAGAGATGAGATACTGTTACCTAATAAAAATGCCATAAATAGAGAATGATGAACTACCATGGGAAATGAATGCATAGAAG
AGGACATGCTGGAATGTGGGACAGTAAAAATCACTTAAACTTTGCGTGACCTTGAAGAAAGTCACGATGATCTGTTTTTCCAGGTCCCT
CAAACAGTGAGATGTGGCTGTTTCCCAAGTCTTCCTCTCCAGTGTAAAGGGTCTGAATTTAGACGCTTTGTGAGTCTTCCTTCTTTCGA

FIG. 3D

CAGCCTGGAGTCTCTCTTGAGTCTCAAGGCTGCCTGAGTTCCTCTCTAACATCCTCTAGGCAGTATCAGCTAATGAGACAATGAATTCC
ATGGAGGCAGCAGTGGGAACAGAAGTACCTCTCTTGGATAATTTACAACACTGGTGAGCAGAGGGTCAGATCACCCTGGGGTTTGTGTC
ACAACCAAAAAAGTGGCTGTGGCACTGAGTTCTTGGATGGTTTTCTACAGCTGGTCCAGATTTTCCATGGGCTCACCTTTAAATTAAAA
GAATTTCTGCACTTTGAAGAATTTGAAAACAAAGCCATGTGTGAGAATATGAGATCCACTCATATGCCCTTGCAAGAAATAGGTTGCAT
TCCTTTTTCCGGACTTAAAAAAAAAGCACCCCCTCTTTCTTTTTTTCAGAAGGCATATATGTAAATGATTCCAAATTAATCTTTAGCAT
GTGCCTATGTTGTTCTGATTTACTAAACTTTAAAAATATGTCCATTGTTGTCTGTTAACAGCTTTTGGCAACTTTTTCAGAGATTGAAA
TATGTGAGCAAATTAGAGAAATGAGTACAATTATTAGCTAGTACCATTCAACAAGCGCTAAAGATACAAATACCTCTACAATACATAAA
AGGAATGATTATAGTAGATTTTATAATGCCATATAAGGTTTCTTATTTAACTTCATTCTTAATTCTCAAAATAAAATGAAATTACATAG
AAGCAAAGTAATATAGTTACCAGAATAGTATTTTTACATGTCTTTAAGTGTATGTTGTTGTTGTTGTTTTTAAGGTAATTATGTGATGT
TGTGGAAAGAACAGAGACCTGGGTTAGATAAAATTCCGGTTGTCTACCAGATTGTGATAGTGAGCAAATTACTTAACCTCTATGATCCT
TATCTTATTTATCTATGAAACAGGATTGGTAATACTCATATCATAAGGTTGAAAGGATTAAATGAGGCACTATGGAAAATTTCTAACAT
GGTGGTGCCTGGGACAGTAGAAGATGCTTAATAAAGATAGCTTTCATTATTATTATTAGCTTTTTCAGGTGATGGTGATTGTAAATGTT
TAGGTAATTTTTTAAACTTTAGAAATAATTGATTTTCAAATGATTAAGACTGCTTATTTTAATCATTTATTTTTATCACCAGATTTATT
TTTATTACCCAAAATGTCAACGACTGTCATAAAGATAAAAATTAATAATAATTGGCCAGGTGCGGTGGTTCACGCCTGTAATCCCAGCA
CTTTGGGAGCTGAGGTGGGTAGATCACAAGGTCAGGAGATTGAGACCATCCTGGCTAACGCGGTGAAACCCCATCTCTACTAAAAATAC
AAAAAATTAGCTGGGTGTGTTGGCGGGCGCCTGTAGTCCCAGCTACTCATAGTCCCAGCTACTCAGGAGGCTGAGGCAGGAGAATGGTG
TGAACCCGGGAGGCGGAGCTTGCAGTGAGCCGAGATCGCGCCACTGCACTCCAGTCTGGGCTACAGAGCCAGACTTCATCTCAAAAAAA
AAAAAAAATTAATAATAATTTAAACCCGAAGTATGAACTGAATTATTTCCCTTAGTAGCACATCACATAGGCTGATGATAGTTTTGGTG
ACTGGTTTATCTATTCTTCCTAAAAGCAAACTGTTGTTAGATGGATGATCACTTGCATGTTGTGACTGAACTCAGCAGTTGGGTTTTAT
TTTTTATTTTTTATTTGCTTCAGTAGCATTADCCTTTCCTACCAAGATTCGAACAATCCATTTGCCTTTTTTTCCCTAAAATCTCTCAT
ACATTGTAAATACTACATATTGGCTAAATATTTCCTGGACAGACATGAAGGACACATAAATCAGTCTCTGTATGATGTTTCTCACTGTA
ATGGAGTTTATCTGGCTCAAGACCAGGACATTTATTGCATATCAGGTTTCTACAGTTCAGGCAAAAGTTTGAGGATAAGGACTTACTGC
AAAAAGTCTTCTATTGTTCTCAACCATTTTCTCGCTTAGCACATGCAGAGATTTGAAATGGTCCGTGGTACAGTAGTTGTGTCTGTATA
TTTCTCTTGTAGAATATTAGAACAAGGGATTTGCAGTTTACAGAGAAGAAGGCTTGGCGAGGTGTTTGGAAATACACTCAGAAACCTGA
GGAAATTTGTGGAAAGAGAGGCTTATTATTTCTAGAAATATGCTAGAGTWCGTTTTGATTGTGCACCTGAGGAATTAATAGATTAAGTA
GTTTTATAAGGACTGGGGTTAATAGAATACTGGCAGTGAAGTTTGTCTTAGGACTTCTTAATTGGATAATCAGTGAAGTCACCAGATCC
CAGTTAGAGACAGTTCCAAGTTTTACAAAACGCAAGATAACTGTCCAAGAGCTGTAATGGCTTAATCATCTTTGAATAATACCTCTCAC
TGAAGCTATATCATAAGAAATAAAAATCTACATTTTAAAAAATTGGCTGTAATCATAGGGTGACTAACTGTCCCTGTTTACCCAGGACT
CAGGGTTTCCCAGGCTGAGGGACAATGGGTACTAAAACCAGGACAGTCCCAGGCAAACTGGGACGGTTGATCACCCTACCCAATGGCCT
CATCTGTCTCATTAAAATATCTGGATTACTTCGTGCCTCAAAAATATCCTCGGCTTACCTGACTCTAGACAGTCAAGAAGCTTTTATTA
ATTGTCTAATGTATGCCACTTTCTGGAGGTGATATTGTTCAACTGATAGATGAGCATCACTGATTGAAATATTTTGTGGTTTTCATGCT
TTGTATCTTGTGCTGATAGCCCCACATGGATATTTCTGTTTCCAAGTTTGTGTCACTTCTGGAGATATTAGCCTGAACTCAGCAAAATA
GGATGATCAAAATGAACCTTTCCAGTGAATTCTGTCCTTCTTGTGCTGTTGTCATCTGACTTAGATATACTGGCCGGGCGCGGTGGCTC
ACACCTGTAATCCCAGTACTTTGGGAGGCTGAGGTGGTTGGATCCCTTGGGATCAGGAGTTTGAGACCAGCCTGGCCAATATGGTGAAT
GAAGCCCTGTCTCTACTAAAAATACAAAAATTAGTTGTGCGTGGTGAAGTGTGCCTGTAATCCCAGGTACTCAGGAGGTTGAGGCAGGA
GAACTGCTTGAACCAGGGAGTCGGAGGTTGCAGTGAGCCCAGATCACACCACTGCACTCCAGCCTGGCAACAGAGTGAGACTCCATCTC
AAAAAAAAAAAAAATTAGCTGGATGTGGTGGCACATGCCTGTAATCCCAGCTACCTGGAAGGCTGAGGCAGGAGAATCGCTTGAACCCA
GGAGACGGAGGTTGCAGTGGACGAGATCGTGCCACTGCACTCCAGCCTGGGTCTCACAGCGAGACTCCATCTCAAAAAATAAAAATCA
ATAAAAAATAAATAAATACATAAATAAATGAACACATAAATTAGATATACCAAGAAAAGTATAAAAAAGTCTTGTGTGAACATAAATGA
AAATTGGCCAAAATAGGTAACAGACAGGGTCAGGCGTGGTGGCTCATGCCTGGAATCCCAGTACTTTGGGAGGCTGAGGTGGGAGGACC
ACTTGAGGCCAGGAGCTCAAGACCAGCTTGGGCAACAAAGCGAGACCTCATCTCTATGAAAGAAAAAAAAATTTAAAAGACGTAATGAA
CAACTTGCTTGCCTTCCTGCCTGCCTTCCCTAAAATACTAAGTTAAATGCAATACATGCCCTGACATTGTAGTTTGCTTTCACAAAGAT
TTACTGAATACTTACTCTAGGCTAAACCTTGTGCTACATGTTGGGGCTACAGGGATGAAAGARAATTGGTCTTGCCCTCCAGGAACCTT
TCATTTAGTACAGAGATTTAGTGTGTGCTGGTTGGTCTCTGTTCTCCCCCTCTCCTCCAGATCTATTCTCTATTTCTTCCCCTCTCCCT
GCCTCCAGGAAGGGGGGCTGGATCACTGTGGCTCATTGCTCTGTGGCTTCTGATTGAGTTCAGCCAATGGGAGGCATHATTTTGGCGTG
GCAGCTCTGGCTGTTCCTCTGCAATTGCAGTTCCCTCCTCCAAGGCTCTGGCTCTCACTGGGTTCCTGTATCCAATAACAGACTCCCTT
AACTGCCCACTTCTGAAAACAGTTTCTGCATAAAGCTATTTTCATAATTTCCTCTGATGTGCCTTCTGTTTCCTGTGTAGACCCTGATT

FIG. 3E

CAATAGGAAAATAAATTATTGAAATAGAGGAAGAGACAGGTAATAATAGAGGTATACACAAGTAGAATGGGGCAATAAATGGCGCATTT
TCGCACCATCAAGAGTGCCCATGTAACAGAGATAAGTAAATGCATCTTGAGCTGAACACTGAAGGATAAGAAACAAAGGGGAGAAAGAC
CTAGAAGGGGCAATATACAGCAAGGAGGGAAAATAAACTACTGTGCATTCATGCCAGTGTTAGCATTTAGGACATCTGGAAGCTAGAGG
TGGAGTGGAAAAGGAGAGAGTGATAGGAGCTGGGGTCAGAGAGTTTCAGGGTGGGGAAGGTCTTGCAGGACCTTGTAGGTAATTGTAAA
GCATTTGGATTTTATTCTGAGGGTCACTGGGGTGTCATTAGAGACTTTTGAGCAAAGAGGTACATGCTCTGACTGAACTTTATTCTGTG
AACAATCAGAATCAACTAGATGGATTTAAGTATGGGTATACCATGAAAGAAAATTACTTAAGATCCTTGCTACTCAAAGTATGAGCCAG
GACCAGCTACACTGGCATMAGCTGGGAACTTGTTAGAAATGCAGAATCCCAAGTCCCCGAGACAAACTGAATCAGAACCTGCACTTTAA
CAAGATCCCAGGTGGCCCATTTGTATGGTAGAGTTTAAGAAGCATTGGTTTAAAAGATCCCTCTTGATAGGAGCATGGAAGATACATTT
GAGACAGAATAGACAAGTCAGAGACAGGTGGGAAGGGCCTAAAACAGGGCAGAAGTAGGGAGGTAAATGAGGAGACAAATACAAAGGAA
GAAAATGCACAGCACAGTGTAGACAATTCCTAAATACTTAAAAAAATTTTTTTGAAATAATGATAGATTCACAGGAGGTTGCAAAGAA
ATGCGTAGGGAAGAACAATGCACCCTTTACCCAGCCTCCTCCATCATTAACATCTTATGCAACTATATTATAATATCGAAAACAATCAA
GTGACATTGCTACAACCCATAGAGCTTATTCAGATTTCACCAGTTATTAGATGCACTCGTGTGTGTGTATGCATATAGCTCTGTGTAAT
TTTATCATATGTGAAGCTTTGCTACCACAATCAAGATATTCAAGCCATTAGCAGAAGATTTTCTGGTGTTACCTCCTTATAGCCACACG
CATTCCTCCATCATTAACCCCTGGAACAACTAATCTGTTCATCTCTATAATTATTCTATTTCACGAACATTTTGTAGATGGGTACATG
CAGTGTGTATCTTTTGGGATTGGTAACAGAGCAAGACAGGATCTCACTCTGTCACCCAGGCTGGAGTGCAGTGTCGTGATCTTGGCTCA
TTGCAGCCTCCACCTCCTGGGCTCAGGTGATCCTTCCACCCCAGCCTCCTGAGTAGCTGGGACTACAGACACACGCCACCTCACCTGGC
TAATTTTTTGTATTTTTATAATGATGGGGTTTCACCATTTTGCCTAGGCTAGTCTAGAACTCCTGGGCTCAAGTGATCCAACCGCCTTG
GCCTCCCAAAATGCTGGGAGTACAGGCATGAGCCACCACCTCCACCAGCTTTTTCATTCATACTTTCTTTGAAGTTCATCCAAGTTGTG
TGTATCAATACTTCACTCCTTCCAGTTGCTGAGTAGTATTCCATGGCTTGGAGGTGCTAGAGTTTATTCATCACATTCAACCCATTGAA
GGMCATTTGGGTGGCTTCCAAGTTTCCAGTTTTGGGCTATTATGAACAAAGTTACTATGAACATTCATATACAATGGATACTTTTTGTA
TGAATGAATGGAATAGAATGGATAGGATTTAGTGATCAGCTATGTGGGATGAAGAGTGGCATAAGTAGTAAAAAGTAACCCTCAATGCA
ATGTGCAGCCAGCAAGTACCACAAAAAGAGTTTATTTTGTTTCATACATATATTTCTATATATACATACACACACTTTATTAATAACCA
AATAGTATCCTTTTCAAATGAAAACAGTAATTTAACATAAACTATGAACTTAAAATCTAAAGTAAAACTTGACAACAGTGATGCAGAAT
TTTTTGCTCCTTAGCTCAGTTAGGTCTGTGTTCTTATCTTATGACCAGGAAGAACTAGGTACCCTGACATCAAAGAATGAGTGGCATAG
AATTTATTAAGCAAAAAGGAAAGCTCTCAGGAAAGAGTGGGGTCCTGAAAGCAGGTTGCTGGTTGCCCCTTCGTAGTTGAATACAAGGG
CTTCTATATAAAACCTGATGGGGCCGAGTTCCCTGTTCGTATAAGGCATGAATTCCTGGTGGCTCCACCGCCCTCCCCCAGTGCGTATG
TGGGACCTTCGTCCACTAGGGACATGTTTAGACAAGCTCCCTGTGCACGTTCCCTTATCTGCACAAAACATGGGTTGGAGGTTCTCCGG
GGACCCTTCCTTTACTTTCTGCCTAAAGCAAGCTGGCTAACTCCTTTCAACAATACTAAAGACATACAGACAATGGTTCTCAGTACAAT
CATTTTAAATATTTAAGTAAACTTAAAATGGTGTTTGTTTTGATTTGACATTTTAAAAGATATCGCTGTTCTAAAAATTCTGTGTTTTT
AGTTGTTTGGGCTCCTATTCTACAATGTGCTATTACTATTAAGCATTCTTGTATCATGGCATTCCTCAAATAGTTTTTAAATTACTTTT
AATTTGAAGAAGGAACATTCTGTACAGTCACGGAAAGTGTCAAAAATGAAAATGAGGCAGGGTGTGGTGGCTCACGCCTGTAATCTCCG
CACTTTGGGAGGCCTAGGTGGGTGGATTGCTTGAGCCTAAGAATTTGAGACCAGCCTGGGCAATATGGTATAACCCTGTGTGTACAAAA
AATACAAAAATTAGCCAGGTGTGGTGGCCCAAGCCTGTAGTCCCAGCTACTTGGGAAGTTAGGGTGGGAAATCCTAGGTGACAGAATGA
GACCTTGTCTCAAAAAAAAAAAGAAAAAAGAAAATGATAAAGGATACATATCAGGAAAACATGCATGGTATTTTGTATCATCTACTTTA
GAGTAATTCCAGTATAGTGGTTTTTTTGTTGTTGTTTGTTTTATTTTTGAGAAAGGGTCTTGCGCTGTCACCCAGGCTGGAGTGCAGTG
GTACGATCTTGGCTCACTGCAACCTCCGCCTACCAGGTTCAAGCCATCCTCCCAACTCAGCCTCCAGAGTAGCTGGGACTACAGGTGTG
CGCCACCATGTCCAGATAATTTTGTATTTTTTGTAGAGATGGGATTTTGCCATGTTGCCTGAATGCCTGGCCTCAAGCAATCCACCCTC
CTCAGCCTCCCAAAGTGCTGGGATTGCAGGCGTGAGCCACCACACCCAGCCCCAGTGTAGTCGTTTTTTCTTTTCTTTTTTATTCTATG
TTTTAATGAATTTACACGTTACCCAAATGTTCCCTAGTTTTTCTGCCTTCCAAGATCACTCTGGAAGAATATTTAAGAATATACCAAAT
AAGAATATGCAAGTCCTCCCCTAAGGGTGGCAGGAAGAACACCCCTCCCCCAGATGGTATTTAGCGCCTCTGGCTGGGAACGGCTTCCC
CATGCTCCTAGGTCAGGGTCCTCTCTTGGCATGACACTACCACCACAGTGCAGACCCACAACAGGGAGAAGGACGGCCACAGTCCCTCA
ATCCCCCTTTTCCAAGATGTGCACAGCCTGACTCCTAACTCCCCACCACTGACTCTAGGGGAAAAACAGCACAGGGCAGGAAACGATTT
TCCATGTCACCAACCTTTCTCTGAGGGAACCTACTGGCCACCTCCCTCTTAGGACCAGCCCATCGTCCACAACGTGGAAGTCCAGCTTC
CGTTCAAATCGGAGTTCTTTCTTCATGACATTTCTTTGCAAAGTCCCGGAACCCACAGCTCTGAGACTCTGGCTGTCCCCCAACCCACC
CCATCTTCCTTGTCCTCACCCCTGGTCAGGAGAAGCCAAAACATCAGTCAGCTTCCCAGTAATCAAGCCTGGCTTTCTCACCCAGGGCT
CGCCCCAGAACAACCACCGGCTTCTTTCAGTGTAGCCAAAAGGCTATTGGAGTCTTCTCAAATGAAAGAGATTTTATCAAAGGCTTGGA
GAAGAAAAGAAAAAGAGGATTATATAATAAAACGTAAAACAACAAACATATACACACAAACAAAAATAAACGTGAGATATGATTCTCCC

FIG. 3F

GGAGTGTTTAGAGCAGGAATGTTCTTGGGCATCTGCCTTCCCCCACCAGCACCCCCCACAAGGCAAGGCCAGTTCACCCTCAGTGCTCA
CTACTTTGCAGTGTTCATAGAATATTTGTAATAATTTTAGGCGGCTCCCTAAAATTTCTTTTCTTTTTCTTTCTTTCTTTAGAGTTGCG
TCCCTCTCGGTTGCCAGGCTGGAGTTCAGTGGCATGTTCATAGCTCACTGAAGCCTCAAATTCCTGGGTTCAAGTGACCCTCCTACCTC
AGCCCCATGAGGACCTGGGACTACAGGTATGCACCGCTATACCCGTCTATCTTTTATTTATTTATTTATTTAGAGACAGAGTCTAGCTC
TGTCACCCAGGCCAGAATGCAGTGACACGATCTCAGCTCACTGCAACTTCTGCCTCCCAGATTTAAGGGTTTCTCTTGCCTCAGCCTCC
CTACTAGCTGGGATTACAGGCTTGCACCACCTACGTCCGGCTAATTTTTGTATTTTTAGTAGAGATGTGGTTTCACCATGTTGGCCAGG
CAGGTCTCGAGCTCCTGACCTCAAGTGATCCACCCGGCGTGGCCTCCCAAAGTGCTGGGATTACAGGCGTGAGCCACTACGCCCAGCCT
ATTTTATTTTATAATTTTGTTTTAGACAAGGTCTAGCTCTGTTGCCTGGGCTGGAGTGTAGTGGTGCAATCACGATTCAGTGCGGCCCT
GATCTCCTGGGTTCGAGTGAGCCTTAGCCTCCTGTTTAGCTGGTACTACAGGTGCATGCCACCACCTAGCTAATTTTTTAAAATTTTTT
TGTAGAGACGGGGTCTCACCCTGGTGTCCAGGCTGGTCTCAAACTCCTGGGCTCCAGTGATGCTCCCACATTGGCGTCCCAAAGTGCTG
GGATTATAGGAGTGAACTACTGTGCCCAGTCTTTTTAAAAAATTTTCAAGAGATTGGGGTCTTGCTATATTGCCCAGGCTGGTCTCCAC
TCCTGGTGTTAAGCGATCCTCCCACCTCAGCCTCCTTGAGTAGCTGGGATGACATTACAGGCACACACTGCCACCACTGGCTCTAAAAC
TTCTTCTGTGCCATTTGTGCACTTCACCCAATTGCCTCTTTGTAGTAATTAATTAGGATCTAGGGTGAAAAAAAGTCAACAGCTATAT
ATAGTCCTCAAAGTTTTGTACGTATCTGAGCAGTCATCAGTTGCACAGTGCAGAGGGATGAACTGCCGTCCCGCCACCTAAAAAGCATT
AGTGACCATCAGGGAACCGTCAGATGCATGCCAGACTAAAGCAGAGTGAGGCTGTGCTGGGTGCTCTGTCTGTGGCTGCCCGTGCTCTC
ACTTCCCTGTCTTGCTCTGTGCCTTTGGGAGGTTGACCCTGAGTTGGCATCTCAGGGTCTCAGTCTGCTGGTTTCCTGSGTTCCCCTTG
AAGGCTACTGCTCCCACAAGGCAACCACGGTCCCCGCTCTGGCTCTCACTGAGCTCCAGAATCATTGTTTCCTCCCCTTACCCAAGTGA
GAATAATTATGTTTTATTCCAGAACCCTGACAAATGAAGAGGCCTAAAAACCCCCTAGGTATTATCCGATCTTGGTGATCAGGGAGGTG
TTTGTTTTGTTTTTTAATGCAGACACATAGTTTTAAAAATTATTCACTTCATCTACTGTAAGAAAAGTCATATTAATTCACAATTTTGA
TTAAAACAAACAAACAAACAAACAACTTCTGTGACATTTTGGCTAACAAGTGGTTCAATATTAAAGCTTTGTCCACCAGGTGCAGTGGC
TCATGCCTGTAGTCTCAGTGCTTTAGGAGGCTGAGGTGGGAGGATCACTTGAGGCCAGGAGGTCGAGGCTGCAGTGAACCATGATCTCA
CTACTACACTCCAGCCTGGGCAACAGAGTGAGACTCTGTCTCTAACAAACAAACAAACAAATAAGTATAGTTCTTTCAAGCATGGCAGA
CAATCTGTCTCCTTTGGCCTGGGTCTCTCACTGCCTTTTAGATAAAAATCTGGCAATAACCAAAGAGTTTTCATAAGGCCTGTTGATCT
ATTTATAAGACATGCATATAATTTACTTGACCATTATAATACCATTATAATAATCTAAATCTATTTTCTTTATCGTCCAATAATCCACA
GAGTCAGCACACAAGGATTCTTTTTTCCATATATAGGCTGAGTATTCCTTATCTTACATGCGTGACGCCAAAGTGTTTCAGGTTCTGGA
TGTTTTGGGATTTTGAAATATTTGCATATACACAATGAGATATCTTGGGGATAGAACCTACATCTAAACACAAAATTCATTTATGTTTC
ATATACACCTTATACACGTAGCCTGAAGGTAAATTTACACAATATTTTTAATAATTTTCCACATAAAACAAAGTTTGTATACATTGAAC
CATCAGGAAGCAAGGTGTCCCTGTCTCAGCCACCCACAAGGACACTCTGTAGTTGTCTTTCATTCCTGATTCCGAATTTATACGCTACT
GACAAGCAATCATTTTCTTACACTTATTCACACAAGAGCACTTAGTAAAAAATATGACATATATATCTGGCATGCTCAGAAAAGCTATT
TTGCAGCAGAAAGGAGCTGGGAGGGTCCTTTTTTTCCCTTGGGGACACGGAATAAATTGTGTATTATGTGCCTGCATTTTGACTGTGAC
CCCATCACATGAGGTTAAGTGTAGAATTTTCCACTTGTCTCTCTGTGCTTAAAAAGTTTAGATTGGCCAGGCATGGTGGCTCATGGCTG
CAATCCCATCACTTTAGGAGGCCAAAGCAGGTGGGTCATTTGAGGTCAGGAGTCAAAACCAGCCTGGCCAACATGGTGAAACCCTGTCT
CTACTAAAAATAAAAAAGTTAGCCTGGCATGTTGGTGCATGCTTGTAATCCCAGCTACTCGGGAGGCCGAGGCAGGAGAATCTCTTGAA
CCTGGGAGGCAGAGGTTGCAGAGAGCAGAGATCACTCCATTGCACTCCAGCCTGGGTGACAAAGCGAGACTCTGTCTCAAAAAAAAAAA
AAAAAAAAGGTTAGATTTTGGAGCATTTTGGATTTTGGATTTTGCATTAAGTGTGTTCAAGCTGAAAAGAAAATCCGATTTGCTCAGGA
CAAACTTAACAAAACAAGTGAGATATTCCAATACTATATATATGCTCCTGTTTATATTTCCTTAATTAATTTGGACTTGGAACAACTTG
GCCAATTATGGATTAGAGGATGAGACTTAAATGTTACTGTACAAGGGATAGAACGATTCATTCCTCTATGTTATCAAATACTTATGGTA
TTTTMCCCATCCTGCTGTCATGCAGATCCAAGAACCAAATTAAAACACATTTGCCGGGGTCATAATAATGTGGCCAGAATTTAAAGAAA
AACTTGATTTTTAATTATGTATGATTTTGCTTGTTTAGTCTACCGATTTCTATTTGCTTTAGCTTACTCAAAAATAAAGCGCGGCACTT
CGAAGACTCAATAGTCTTCCATTCATGTGGGCCTTTATAATGCACGGGCCCAGATGCAATACATCTGGCGGTCTGCTTGGGTTGGCCAC
TGGATTGAAGGAGGCAGAGAAGTCTGGGATGATTCCCAAATGTCTGGATCTGGTGACAGGGAGATATGGCAGGGCGAGCTTAGGGGAAA
AAGCTGGGTTAGGAACTGTTGAAACTGAAATCCCTGAGGSYTKTGCCGACAGAGAGACAGCCGGTAGAAGGTTGTCTTTGCCTGTCTGT
GGTTCCAGGTAACTTCATCGAAAGAGAGTTTCAGGCAGTAGAAATAAGAGCACCCAGGACAAAGCCCCAGGGAAGAGAAACATCTGACG
GAGGACAGAGGAAGAAGGGTCAGGAATGAGACTGAGCAGGTGTCATGTGTCTGACACCAGAGCCTGACACATAGTACGTAGTAGACACT
CAGCAAATACCGTAACAGAGATGAATCCAAGGCTGGGGGAGGTGGCTCACGCCTGTAATCCCCACACCTTGAGAGGCCTAAGTGGGAGG
ATCTCTTGAGTCCAGGAGTTCGAGACCAGCCTGGGAAACATGGTGAGACCTTGCCTCTAAAAAAATAAAAATTAAACATTAAAAAAAGA
GATGAATGCATAACCTGGCTGCTGGAGCCAACATGGGTTGGGTGAGCCCACTCTTACCAGCAGCTAATCAAAAATTTGCCTGGAATTCT

FIG. 3G

GAGGCTCCTGTCCTACGTCTTGGCTGCTCCTCCCAGATCACCTTCTGGCCGGTCCCAAGTCCACTTCCCGTGCTCCTTGCTCCCTTCCT
CCTGGTCTCCCTCACACTTTCCTTTCCTACTCCCCTTCCCTCTGTGGCCCTGGCTCAGCCCAGCACAGGGAGAGCCCTGTGCCACCTAT
TACAGCTCACCTGCACCTTTGCATCTTTCAGAAAGGAGCACCTACAAGATAACCCACCCCCCACCTTTTTTTTTTTTTTTTAGTAGTA
CAGATTGCCTCTCATAGCATAATTGGGCTTCATTATTATCCTTAAAGACCCTCTTTCTGTGGCGGATTGGGATGGATAAAATAAAGAAG
ATCGAGAGGTTGAAGAACCCATCCTGTTTTGCCAGTGAGAAGGGGATAGAATTAAAAGGATTAGGAGGGCTCAGGCATGGTGGCTCCAG
NGTGTCATCCCAGCTACTCAGGAGGCTGAGGCGGGAGGATCACTTGAGCCCAGGAGTTGGAGACTATAGAGCACTATGATTACACCTGT
GAATAGCCACTGCACTCTAGCCTGGGCAACATATCAAGACCCTGTTTCTAGGGACAAAAATATNNTTTAATAAATTTAAAAATTAAGGG
AAAGGTAACCACATCCTGCTACAAANAAAAGAAGNTGGAGAGGTANGANGAGGACCAAGAGCTAATGGCATCATTTACACAAAAAGAGA
TGCTTTAAAATCAGTTGCTCATCCAATTCCACAAGGACAATAAGTAAGAAAGAGGATAGAAAGTCACCGGTGGATTTGGTCATCATTGG
CTTCTTGATGACTTTAGCAACAAAAATTCTTGTTGGTAGTGAGAGTTAGACCCTGGTGGACTGGGTAGGGGGTTCCTGGATCATGAGCA
AAGGCCTGTGCCAGCCAATGGCCCCCACTACACTCTGCCCCGGCCTTTCTCATCTCAAAAAATGGCATCCCCCATCCAAAGCTCAAGTC
AAGAATCCAGCAGCCACCTTTGATTCTGCACTTCCCCTCACCTCACAGTCCAGTCCCATCTCCAAAATAAGTTCCAAATYTCACCACTT
CTCATTCTCCAAAGAGGMACMATTATCTCTTTCCTGGTGATTAAAACAGCTTCCTAACTGGSTTCCCTTCTACCTTGCTTTCCCATAGT
CCATTCTTCTCAGGACAACAACAGTGGCCTTTTAAAACCAGTGCATTATTGTTGCCCTTTGGGAAATCCTCCACAATTATCCAGTCTTG
CTTCAAAAAATGTATGTATTTCTGACTTTTTACCCTGCCCTACTTACAGGATATGCACATTTCTGATCTCCAGCCAATATCACACTTCT
TCTCTCACTGCACTCTGCCACACTTGGCCAAGTTTGTTCCCACTCCTCTTGCACTTGCTCTCAGATCTCAGAAGAGGCGTGCTCCTTGT
CTTTCAGGCCAGCCGGCTTCACACATGTGCCACGTGCGCCCCTCGCTCAGAAGGGATCTGTACTCGGTTTGGATCTATTGTTGCCATCT
TGAAACTCTTAATACTCTTTGAACACGGGGCCCGTATTTTCATTTTGCACTGGGTCCTGAAAATTGTGTAGCTGGCTCTACTTTCAGGG
ATTGTATCAGAAGTCTCCTCCTCAAAGAGGCCTTCCTCGGCCACTTATCCTCAAGTAGCTCCTCCCCTTCTAAGTTACTGGCTATCCCA
TCATTCCCACTTAATTTTCTTCATAACAGTTGTCATGCTTTTATACATTCTGGCTTCTATATTTATTTGTGTATTGTCCAGTTCCCTCC
CTTTGGAACGCAGCGTGGGCACCTGCAACGCAGAGACCACTGTATCCCCGGTGCAGAATGTAATGAGTGCCTGATACATTTGCCGAATA
AACTATTCCAAGGGTTGAACTTGCTGGAAGCAAGAGAAGCACTATTCTGGGTAAAATGGAAATTTTAAATGTACTTGATATTTATATAC
ATCCTAATCAATAATTAAATTTGTGTAGTGCTGATCTAAACAGATAAATTCTGGCTTCATGATGATGGTGAAGTGGAATATAATTTTCT
CATTTTGTATTCAAACTAGATCTTTTTCATGAAAGGATTTGAAGTCTAGATTCAATGCCTACTTTTGCTACTTATGTTATATGAAACTA
AAACAATTATTTTATTGTATTTTTTTGAGATGGAGTCTTGCTCTCGTTGCCCAGACTGGAGTGCACTGCTGCGATCTCAGCTCACTGCA
ACCTCTACCTCCCAGGTTCAAGCGATTCTCCTGCCTCAGCCTCTCGAGTGGCTGGGACTATAGGTGCGTGCCACCACACCCAGCTAATT
TTTGTATTTTTAGTAAAGATGGGCTTTCACCATGTTGGCCAGGCTGGTCTTGAACTCCTGACCCAAGTGATCTGCCTGCCTCGGCCTCC
CAAAGTGTTGGATTACAGGCATGAGCCACTGTGCCTGGCAATAATTTTAGTTTAGTCTGAATTTTTTTTTTTTTTTTGAGATGGAGTCTC
GCTCTGTTGCCCAGGCTGGAGTGCAGTGACGCTATCTCAGCTCACAGAAACCTCCGCCTCCTAGGTTTAAGCAATCCTCCTGTCTCAGC
CTCCCGAGTAGCCAAGATTACAGGCACCTGCCACCACCCCCAGCTAATTTTTGTATTTTTAGTAGAGATGGGGTTTCACCATGTTGACC
AGGCTGGTCTCAAACTCCTGACCCAAGTGATGTGTCTGCCTCAGCCTCCCAAAATGCTGGGATTACAGGCCTGAGCCACTGTGCCTGGC
CTAGTCTGAATTTTTTAAAAAGGTTATTGGTCTACCTTCCAATGACATTGCACTCTGTGTGGCTCAATAAAACATTTTCATTTATAATA
ACTAATTTGACCTGCTCAGCAATCTCTAAGCAAGATAGAGTAGCTGTAATTCTTCATTTTACAGGTCATGTCAAATCATTTCGTACATT
CCAGCTATGTACGAGAGCTTGGTGAGAATATGTGAATAATAATCACAGAACTTCAGAGCTGGGAGTAACAGCTGGAAATATTTCTTCCA
ATAATTGCATTTTTTATGAGAGGACGATGAGGTCCAAGTGGACAGGACCATGAGACAATCGTGTGGCAAGGAAGTTGATGCAATTTGAC
CTCTTAAGTCAGTGATCTTTATGTCCATCGGTCCTTTCCAGCAAGTGAGTTAGCCAACCTTTGCCTGCAAAGGAGGAAATTTTTAATTG
AGGATTTACACTCTGCTTCTAAAATTTTGCTTATTATTGTGAATAATTTTCTTTAAGTTTATTAAATGAATGGCTGAATAAATGGACAT
AAGGAAAGAAGGAAGGGAGGAAGGAAGGGAGGGAGGKAAGGAAGGGAGGGAAGGAAGGAAGGAAGGAAAGAAGGAAGGAAGGAAGGAAA
AAGAAGAGAGGAAGGAAAGGAAGGATAAGTCTGATGACAGCTGCTATTATATTCTACGTGGATAATTTATTTAGATCTTTATACTTTAT
CTTTTGTTTTACTTCTCTTATGCATATTCTCCTCAACTTTTTTTCAGTGGGCCAGAGGAGGAGGACTGCCTCTTGTGACTGTGGAAGGA
CTTCTACCAGGCTAACACCCCTGGCCTCTCACCCTCCCATTTCTCACCCTGCAAAGCAGAGTGCTATTTGATTCATGTTCTTAGTCTGT
GGATCTCAGTTGAGGAGAACTCGTTAGAGATTTGCCCTCTTTCTGTCTTTTTGAGACCTTACTGGTGCAAGACAGCAAATCCTAGCTGG
TGTCTACAGGACACATGCACTCTTAGGTTACATAACTGCAGGGACCACTGTCATTGTATCCTGGAGCTGGTTCTATATAAGACACAGCC
TGAGCAGTATATAGGCTTCCTAGTCTGCTCCTGGCCAAATGTCCCAGTTGGAAGCCCAGAGGTTGTCTGGCTATGCCAGTGGCAGGATG
GGCAAGTCTAACTCAAGGGTGACATATTAGCAAGACCTTTATGGCCATGCATCTAAGATGCTCTGTCCAAGCCTGAACTTAGCAACAAT
AAACCTGACATTTTGAAATCCATCTGATTCCTCTATTTTCCAGTTGATGCCACATGCATCCTCTTGCCATCTTTCTTAATTAAGATGAC
TTTGCTTCTAAATCTCCTTAATTATCAAGCAGCTATCTACAATATTTTGTAATCCCCTTAAATCTTGAGCATAATGATGTCATAATTAT

FIG. 3H

GAAAGTGMCCGGWTTCACATGAAGTATTGCTTAATCTTAAGAACAAAATGGCAGCTGTGAAAACAGATGAAGTAATTAGAGGAAGAGCC
TTTTTGGAAGCTTCGAGATATTTTCAAAGTAATTAGTACTAGTTAGCAATAAAGTTCTGTTCTGAGAAATTGCTCTTAAAGGAGGAACA
TGGATTAAAGAAAAAAATCTGCTACTAGGAAGTAAGCCATCTTCCTATGTGTGTGATTGGTTTTGCTTCCTGAAAACTGGTTCCGTTTT
CAACAAAATTTGGGTCTGTTGAAAAAGAACACGCAGATGCCAGCCTTGATGTCAAACGGGCCCAAACTTGGACAGTGGTAAACTAATGA
GCAATGGTGCACAGAGTCAGGGTAAAAGCTGGACAATTTCCTATGACCAACTTTTCCAGGACTCTGCTCTGCTCTTCCTGAGAAAAATA
CCCAAAGTGCTGCCTCTTCCATTGGCCCAACCATGCATCTTTCAGGATAGGMCACATCTGTTTATAGGTGTGGATTGTAGTTGCTCATA
AGTGACATTAGGCTGTTTAAAATAATAATAGTTCGAGTTTTGCTATGAGCTGATCTGTTTTCCAAGAGAGCTAAGAGTTTTCCAGCTAA
AAGAGGGAATTAGTGGGTAATCAAGGCAGCTGACATGGGGTGTGGCTGGGCCTTGAATGTGTGTCACTCTCTGTGCCCAGGCAGAGCAA
AGATAAACTCCAGACTGCATGTTGCTCAGAGACCAGGACCAACGTCATAGGGCGCCTAAAAGGCAGGTGGCCCAGTTCAGAATTGTCAA
GGTCTGACCTGCTTGGACAAGTGCTGAGTACATAGTAAGGATGGATTGGCTAGTCTCTCAAAACTTGCAAACAGGGCGCAGGTGATCTT
GAGATTTCAGGTGCCGGAGAGACCCATCGTGTAGATTCCAGAGTTGGCTATCATGACTAACAGCTGTCTAAGTTGTTTTTAAATGAATC
ATTAAGGGCTACATTTTCAGTTCAGCTAATCAAGTAGCAAATTACGGTGGGTCTAAAATACTTATCTATTGCATTATGTATATGCTAGA
CTTTATCACTTTAGTTGGTTATATCGCTTCATATACTAACAGTCAAAAAATGCCAAACGAGAAAACAAACAAACAAAAATGCCACATGA
CTGTGTAAATACACTTTTCAAACTGTTTTATCTAAGAGTTTACTCACTTTCACATTGTGGCTTATAGTATTTTCAATCTAAGAGACTAA
TTTTGCTTACATAGGAAACTACATATTTTAAATTGAAAATTAAAAAAATATTTTTAAGGTTTTAATGAGTCCTATCAAAACACATTTGT
ATATAGGAAGGTAGCCCAAGGTCACTGTTGCCAATTGTGTACACAGCCTGCCCTMTAGTGTTTTCTTCTAAACAGCACCAAATTTTAGA
TCATAGTTGTAAATCTCAAAATGTTGGGTTAATAGGATTAAACACTGTGTCATCAAATTGATAGGACACAGCTAAATCCCTGACACGGA
TGAAAATTAAAGCAGAGAAAAACGAAGGTCCTTCCAGAAGCTGGTGGCAACTTCACTGGGGAGATATTGCAAAGTTAGTGGTAAATACA
CTATATTAAAAAGTTTTGTTTTGTAAATAGAGTAATGATAGAAGAAGAGTTAGTTGAAATGATGTATGTAAAATGTGATAACTGCATAA
TTACTAGTACAGTTGCTAGTTTACGACTGTATTAAAAAGACATTCCAAATGTTGATCAAATAATGGAGGTTTCTGTGGTTGTTTTCTTT
TTAAAATAGTAAATATACGTAAAGCAGATAAATATCCCCTTTGTGGGAGTTAAAATAATCTAACTTATTTTATAGTTTTAACTTTATTA
AAGCATACGACTATTCTAACTTATTTAACTTTTCTTAGTAAAGTTTTAACCTCTGTATTTAGAATATTTGTAACTAATGTGTATCGAAT
TAAACTCAAAGGGAAATTCATTAACTGAGAAGAAAAAATTTTAACTGTGCACTATTCACATAGCATAATGGGTTTTATAAGGAGTATGA
GAAAAATGTGTGTGGTTGGTTTTGCTTTCTTTAAAAATAATAGCGAACCACGTAGGTAAAAACTCACTTGAGAACATAGACTTTTGGAG
GGAAATGCCAGGTGTGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGGGGGCGGATCACCTGAGGTCAGTAGTTCGAGA
CCAGCCTGACCCACATGGAGAAACTCCATCTCTACTAAAAATACAAAATTAACCGGGCTTGGTGGCGCATGCCTATAATCCCAGCTACT
TGGGAAGGCTGAGGCAGGAGAATCACTTGAACCTGGGAGGTGGAGGTTGCGGTGGGCCGAGATCACGCCATTGCACTCCAGCCTGGGCA
ACAAGAGCAAAACTCCGTCTCAAAAAAAAAAAAAAAAAAAAAAGAATTTTGGAGGGAAAAAAATCCCTCTAACAGATTCGAATTAATTCT
GTGTTTCGAGATGTTTACAAAATGAAGCTTGGACTCTGAGAGGATGTGATCTATCCTCTCCATTGCATTGAGTTTCAAGTACTTCACAT
GGCGGGCTTTTTTAACTGTCGTGAAGTTTAAACCAAATAGGGACTAGAATTTGTTTGTTTTTTTAACTTACATTTCAAGCTTCCTTATG
TCTCAGGCACATTAGCATAAGTTGTCTAAAGTCATAAGGAAAAATTGACAGAAAAAATGCTTTGGAGCCCCAGGTGTTTTCAATTGATGC
CAACAGAAACTAACCAAATGGAAGACATTTGATGCGGGTTTATTTTTCCTTTGCAGTAACAGCGGGAACATGAAGCCGCCACTCTTGGT
GTTTATTGTGTGTCTGCTGTGGTTGAAAGACAGTCACTGCGCACCCACTTGGAAGGACAAAACTGCTATCAGTGAAAACCTGAAGAGTA
CGTTTGGTTTCTTACCTGTGCTGTGTCCTGTTTGCATGTTGGTTGTCCTGCTGGCGTTTATAGTGAGTCGCAGTTGAGAGATAACCATA
TTCGCTGTTTTCACGGTGAAACGTTCTCAAGGCGCTTAAACCAGGTCATCCTGACGCCAAACATCTGGGTAAAAATAGAAAATTCCAAT
CACGTCTCTGCAGGCGTTCACCTTTCCAGATGTTTGTATCATGTAGATACAACTTGCCAGTTTTTTCACTGCATTTTTTTGTATCATCC
AGATGGTTGGTGTCATCTCAGCACAGCTCTAATGAACAGTGAAATACTTTTCTAGCATTTGAAAAATTTAAACCATTAGAGTAATCTGT
GCAATTGTTCTTAAACTAGTGAAAGAATGGGTTATAATTACGTTGAATCTGGTTGTTCTGTGGCCATTAACTTGCAACTTTGCTTGGTG
ATATATACTTTGGGTACTTAATATATAGAAGAACAAATTAGCTAAAATGCAGCTGATTTGGGGTCTGTAATAATCAGAGTCAAGAATGA
GCTCCTCAGTAGGCCACGTTGGCTATTTTGAACAGGGAATGACAATGAATTTTAAACTTACTAAGGGCTTATTAAAGGTGTATAAGACA
CGTCCATTGAGTTATTAAGGAAGCTCGTATTACATGGGATACTTTCTAGGTCTCGTGCCTCCTTATTAGGTAACTGAAGCTGAAAGAAA
GAGAAATTGCTGACTGTGTTTGAGGTCCCCAGCTGGGCACTTAATATAAATTATGAAGAAAATGCAAAATTTTCTCTAATATAAACACA
CTTGAGTCTTAAATGAAAGAAAAAAAATGGATAAATGAAAACAGGGCCTGAGCAAGTGACAAGAATGAGGTTCAGTGAACTCTATTTGT
TTAGGCGCTCACAAGTGAGGAGTAGAAGGTATGGTCCGTGTGGCAGCTGTGTCCATGTGGCAGCTGACAGCTAATTCATTATGATCTGC
TTTCAGAATATGAGCCTATAAGAGAACAATTAAGCCTCTCTTTTGGAGACATGAAAGGTTGGTGAACTTGGTGTTTTGTAATCTGATCA
GATCTCAAAGAAAAAATTGCCACATGTCTTTTAGGTTTTTCTGAGGTGGGGAGATAGATGCAGATGAAGAGGTGAAGAAGGCTTTGAC
TGGTATTAAGCAAATGAAAATCATGATGGAAAGAAAAGAGAAGGAACACACCAATCTAATGAGCACCCTGAAGAAATGCAGAGAAGAAA

FIG. 3I

AGCAGGTACAGTCATTGAAAATAATGTCTGTTCTTACACAGATCTGGACCAGAAATACTGCACTTGTTAGTGCGATTGATGAATTACTT
ATTTTCCTTAGTAATAAATTTCATGGGTAGCTGCTTTTATTTGAGGAAAAGTTTAAGGGAAGCTTCAGATTTCCTTGAAGAACATATTT
CGTGTAGGATAGGCTTCTGCAAGACTCCAACCCGGAATCTGGGGGATTCATCTCTGTTTAAGTGCTGCTTTCTCAAAAATAGATTATTC
TTGGTCTCTTCTGAGTTAGGATATTGAGTCAAAAGTATTTGAAGAGTTTTTTTTTTTACTAGATCAGTGGTCTCCAGAGTTTTTGTTTT
TTGTTTTTTGTTTGTTTCTGTTTTTGAGACAGAGTCTCGCTCTGTCACCCAGGCTGGAGTTGATCCCGCTCATTGCAACCTCCACCTCC
TGGGTTCAGGTGATTCTCCTGTCTCAGCCTCCCTAGTAGCTGGGATTACAGGCTCCTACCACCACGCCTGGCTAATTTTTGTATTTTTA
GAAGAGACGGGGTTTCACCATGCTGGCCAGGCTGGTCCCGAACTCTGGGGCTCAAGTGATCCACCTGCCTCAGCCTCCCAAAGTGCTGG
AATTACAGGCATGGACCACCGTGCCTGGCCCAGAGATTTTTGGTCTCTCATTCCTATGACTAAAAAATTTGTTACCACTCACTCCTAAA
TATATGCATATTCATTTACTCATGAATTAGATACATGAATTGCTACCATTGATATCTCAAGGCACAATATGTATTTAAGGTGAGATTCA
TCATTAGCGAGTGTGGATATAAGTCCACATTTCAAATAATCTTCTAGATATTTTGAAACTTTTAGCCGACTTGCCAGATCTGATTAGAT
CACCATAGTTTTCCCTTGTCACTTGGCCAATAAAGAGCTCATAATGATCAAGTGTCAGCTCTGCCATTTGCTTTTGGTCCGCTTGAGCT
TAAATTATTCATTTTTAAAATCTGCCAAGTTTTTTTTTTTTCAAAGAATCTTGTTAAGCCTCCTGTCCATTTAGTGAAGGTTACTTTA
GTTAAAACTAGATAATAAAATCCATCAGTCTACCTGAGTTCTCTTACATGGCAACTCATTACAATTGGGTGCATGTGAACAGAGCAAGG
GAACTATAGTTGATTCTTCTGGAATGTAGAGGATCCCCTTTTCCCCAAGGTCATCACATACAGTTGGGCACACACAGTATCTGACATAT
GCATCTCAAGAGAGTACCATGTATATCCAATAATGCATCAGCCTAATCACTTTTTCAAATTCAAATAGCTTTATTTAACAGCTATAGCT
TGAACTACATATTTTATCCATGGAGAATACATATTATATTCAAATGTCTTTGGAAGATGTAAAAAATTGTTCATATGCCACAGTATAAA
GTTCAGTAAATTTCTAAATTATAGACATTGAATAGCTTGCAGTTTAATGACATTAATAATTAACATCACACTCAAAACAATGACTTTTT
TAAAAAAGGTTATCTTCAAMCATTMCCCTTAAATCAAAGAGGAAATTAAAACTGTAACAAAAATAATTTGGAAAATATTTTCAATTTTA
ATGTTGAGAGTAAATTACTTTTTAAATKTATTTTTATTTTTTGAAAAATGTTAAGTTGTAAAATACATATAACAAAATTTACCATCATA
ACCATTTTTAAGTGTAACGTTCAGTAGTGTTAAATACATTCATACTGTTGTGCAACCAATCTCCAGAATTATTTTCATCTTGCAAAAAC
TGAAAGTCTATACATATTAAACAATGCCCCATTCCCCCCACCCCAGTCAGATTTTTAATTTAAAAATACAAGTGGAAGTTCTAATATTT
TCTATCTATCCCTCTATCTATAAAGTTGGGGGCCACTGAATTCCAGATTGCTGCTTGCATCTTTTTACTTCTGAGCATCATGGCCTCTG
GGAGTCCGTTAAGCAACTGGAGCCGGGTAGTGTGACAGGCTGACCCCAAAGCTGTGTGTCAGCGTCACCGGACTGGTTGATGTTGCAGC
CTCACCTACTGCCCTGAGTCAGTCAGGGTTCTGGCAAGGAAAGGAGAATGCCTGACCAGCAGCTGCAAACCCTTCTCCCTTTTGGCAGC
AATCAAAAGATTTTGAGGAAATCTAAAATAGCTCCTCATCAGGAAAATGTGGAAGCCCCTCCAGCTGGGATCTTCCCTGGTGGGCTTGT
GAGCCTGGCCATCTGGGAATAGAGACACTAGATAGCACTCATACACTCTTCACAAAACACATTATCACATGGAATGTTTTGAACATCTG
GGTAAACCACTACTTTCATTTTATAGCTAAGAAAACTGGGGTTTGAGATGTTTGTTAATTAACATGTTACTCCAACACTGTAATGAATG
AACTGAGATAAAGTCAGCAGATGTGTGCACGGGGGACCCAGTGATTTTCTGCTTTTCTCACTTCCCTGAACCTCCTGGCAAGGAGGACA
GGGTATACAGCTTTAACAAGAATATTCCACTTTGGGTGGGTCAAGTAAGCAAATGTGGATTTCACTTCTGGCCCTGAAGAATCCAAGCA
ACTAGTAGAATTTTTGTTTATTCTTAAAAATCTTATTGTACAAAAATTCATTGAATTATACTCTTAAGTTTGAGGCACTCAATTAGAAA
GTTAATCGGAAAAAAAAAATCTGTTTAACCCTGAGTATCCCTCCCTAAAATTACTTAAAGCCTAGAATAAAGGTCAGTTTAGACAAATT
ATGAATTGGCAAATATGGTGTTAGCAACCCTAGTCTCCCAGTATTGAGCCCCACCCATTCTCAAGAGTACTGCTCAGTGGTGACCCAGC
ATCCTCACTGTCCCCTTCCTCCACCCCTCCTTATTAATATTTAGTGAGACTATCTGAAACTTATTAAGTAGGAAACCCTAGAGAAGGTT
AGAGTGACTTGACCTCCAAATCAGGTTTTATTTGTATGTGTTTTTAATGAAATGGGGTCTTGCTATGTTGCTCAGGCTGGTCTTGAACT
CCTGGGCTCAAGGGATCCTCCTGCCTCACTTCCCGAGTAGCTGGGATCACAGGCACTAGCCACCATGCCTGGCTCAATGCCAGGTTAAT
ATAGCGCTTTTGATAAACTGTCAACTATAGGAATAGAGTTATAAGCGTGAATCTGCCAGTTGGTACAATGTCTAGCAGGAAACGGAAGG
CGTCGATAGGATATTCCTTAGGAATGTTTACTAGACAGAGGTCTACTTCTTCCATGGCAATGTTTCACTTCCAAAACTTGGGACCTGTG
ATTTGGTAACTGTTTTTTGTCCTGCTTCTGGGCAGTGAATGGAAGGAAGCCTGAGAGATACTAGTTATTATACTGGACTAGTTATAATA
ACAGATGTCTTGCCTATGATAATGGATACTAGGTATAATAATAGATGCCTTGCTTGTTTAGCTCATTTAATGCAAAGACCTTGAGAAGT
AGATACTATTATTCCTATTATTCTTATTTGCAAATGAGGAGACTAAGGCTTATATGTATTAAGTAATTTGCCCAAGGGTACACAGCCAC
TGTAGTTTGGAATTGGGAATATTAGGATTTTGGCTTATGAGGACAATGAGCAGAATATGTAAAATTGGGACTGATTGAGAAAATCCTGG
AGGTATTGTTACTTGCCTTGGAGAAACAACTTTTTTTTTTTTTTTTTGAGACAGAGTCTTACTCTTGTTGCCCAGGCTAAAGGACAATG
GCACGATCTTGGCTCACTGCAACCTCCGCCTCCTGGGTTCAAGCGATTCTCCTGCTTCAGCCTCTGAAGTGGCTGGGATTACAGGCACC
CACCATCATGACCAGCTAATTTTTGTATTTCTAGCAGAGACAGGGTTTTACTATGTTGGCCAGGCTGTTCTCAAACTCCTGACATCAGG
TGATCCACCCGCCTCCAGCCTCCCAAAATGCTGGAATTACAGTGTTGAGCCACTGCACCCTGCCGAAAAACAACCACTTTAAGATGTTA
GATTCCAGCCAAGTGAAGTGGCTCATGCCTGCAATCCCAAGCACTTTGGGAGGTCAACCTGGGCAGATCACTTGAGGCCAGGAGTTCGA
GNTCAGCCTGGGNAAANTGGTGNAACTCCGTCTCTANTANAACATACAAAAATTNGCCCGGCATGGTGGCACGCACCTGTACTCCCAGC

FIG. 3J

TACTGGGGAGGCTGAGGCAGGAGAATCTCTTAAACCTGGGAGATGGAGGTTGCAGTGAGCTGAGATTGCACCACTGCACTCCAGCCTGG
GCGACACAGCCAGACTCTGTCTCAAAAAAAAAAAAAAAAAAAAAAGATGTAAGATTCCAAAATTGTTCTACAAAGTSCAAGGACACACA
CACACTCCTGTCTGGGTCAAAATGTATATTGGCAAGCTGGGGCCCTGGCAGTTTTCTTACGTGGATCATAGCAAATGCTACGTGGCTTA
GCAGCCAAACTTTACAATGAGGACAACKGACAAATCCTAGCCAGGCAGAGAAGATGTGGAAGATTGTCAGTGCCCAGGTGATTCTTTGG
GCTTAATACTCCAGGAAAGGGTCATTTCCATTAGCTCTGAGGCTGTCTTCTTATGGCCAGATCCACTATACTCACTTCATTCCCCTGCA
CGATATCTCGGCATGGAGGGGGCTGGGGTTCAGAAGTCCACACTTGCAGGGAAGCCAGAGGTTTGGGCAGGGGCACAGGAAGAAAGGTC
TGTTGCACCATGGTGCTGACCCGTGAGGCACTCCAGGGGCAGGGCTGAGGCTCGCAGGGACAGGTGCCACTGCTGCTGGGCTCCTCACC
ACCCAGAGCAGGACTTGGCCAAGTACAGCAAGCACCACAAGGGGGAGCACTGGGAATATAAACAAGAAGAACAAAGCTTGTTTATATTC
CCATTTATATTTATTTAATATTACATTATATATAAATATATTTATTATATTACATTCTAATTGCAGAGATGCCATCCTGCGTCTCGGCA
ATTACAATGTAACTCAACGGGAACATTTAACTTGACATACAAGAATTGTACTTTCTTGCAATGTTTAAGGATATACAACAATTAAAGAC
AGCATAAATGAAAGAATTAAAATGTACCAGCTTTATAAACTGTAAAGCCCACTTTCCCCATGCACCAGTGGATGAGAATTGAAGACAGA
CTTACCGGTAAATAGGTAAATCACAGTTGTTCCCAGATCGGGATGGCATCTTCATTGTCAGGTCACCCACACCTAGAGTAATGTCTGTC
ACATAGCAAACACTCAGTAAATACTTAGTGAACAAATGAATGAACAGATGAATAAGATTTACAGTCTTCAATAGGAATCAATCAGTGCT
CTTTTCTTAAACTAAACAGAAAGCTTTGGGGAGATCTGACAGCTGCGAGGCACCTGAAGGAGAAAGAATGAAAAAGCAGTTTAGAATGT
GTACATTTCAAAGGGTGAAATCAACTAAGGTGCACATAGATCATGAAATGGAAATTGGACTTTTGTTTCTACTTTTAACTAGGAGGCCC
TGAAACTTCTGAATGAAGTTCAAGAACATCTGGAGGAAGAAGAAAGGCTATGCCGGGAGTCTTTGGCAGATTCCTGGGGTGAATGCAGG
TCTTGCCTGGAAAATAACTGCATGAGAATTTATACAACCTGCCAACCTAGCTGGTCCTCTGTGAAAAATAAGGTAAGAGAAAAAGAGAG
CTCAAGATTTCACAGTTCTTAAGGCACCTATTTCAGCTTACTTTTTTATTAATTTATGTTAATATTTAGAACGGAGATGCCTGATCTGA
TAGGGGCCTTTTGCTTTCTAGAATCTAATACTAATGTTTACATACCATCACCTGTGTATACGCAATTTATAAGGTAGAGCACCATTCAG
TGGTCACTGAATGCATCTCTTAAAATATCCTGGCTTTCTGCCTTGTATTTGTTATTTGTGAACATGTTCCCACTAGATAGTAAGCTCTT
TGAGGGCAGGGATCATATCTTATTTGTCTTCACTTATGCATTGGTGGCATCCAGTAAATGTTTACCAAATTGCATTTGGAATCATAGCA
TTGCAGTCTCTGATTTCAATCCACATTAATTTTTCCTTCTGGAGGCCAAATATTTAAAGATACTCTCTGCCTCCCAAATCTTACCTTCA
ACATGCTTGCCTCCTTATGCATAACACACACACACACACACACACACACACACACACACCCCTTCATGTCCCCTTTTGCCCTACCCATG
TATGTAGACTGGCATGTTTTCTTTTTTGTACCCTTTGGTTATCTTCTGAGCAGAGGGATCACAGAGGGTGGTGACCTGAATAGGATGAG
CTCTGCCCCACTAACGGCTCCAATTAAGCTAGATTTTTCTCCCCCTTCAAGAAGTGAGCTGAATACAAAATTGAGTGGAATTTCACGCT
CCATATTAGAGCACATACTAATTAGGGTATGCTCCTGGCTTGGCAATGCCATACTCAATTACAAAGGGAGCAACTACTAAGATAATGAA
TGCGCCAAGTTAATTTGCCTCCACTATTAATTGCATCTGCTCTATTTTTAGAGCTACTGTCGCCTGCTAATACACCAGAATATGGTGTA
ATCAGCACCAGCAGGAAGTCAGGAGATATGGGGACCATTCCCATCTGGGTCAGTTGTGTGATCTTATGAACATTTCTTGGGGCTTTAAA
GGTTTGTTTTTGTGGATGAAGAGTCAAGTAAACAGAAGCTGGTAGAGGGAGAGGCAGACAATCCACCCAAATTCTTTTCTTTATTTTTT
TTCATGAGACAGGGTCTGGCTCTTTTGCCCTGGCTAGAGGGCAGTGGTGCCATCTTGGCTTACTGCAGCCTCCACCTCCTGGGTTCAAG
TGATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGGATTACAGGCGCCCACCACCACGCCTAGCTAATTTTTGTATTTTTAGTAGAGACAG
GGTTTTACCATGTTGGCCAGGCTGGTGACCTCAGGTGATCCACACACCTTGGCCTCCCAAAGTGAAAACTTGACCTTTTTAGGCTATTG
GTGGGCAATGTAAACCAGGAGAAATTTCAGATCCTGTTTCCATAGGCAAAGGCAAAGTCAGGTATAAGAGGGTTAAGAAATTATCTTAA
AGTTAATTGCCTCATACTAGCTTGCCCAGAATTATTATTGATTTGAAATGACTACTGTAAGTTGACTTTAAAATTTGCAATAAGAAATG
GTCCAGGGCCGGGTGCAGTGGCTCACCCCTGTTATCCCTAGCACTTTGGGAGGCCTAGGCATGTGGATTMCCTGAGCTCAGGAGTTCGA
GACCAGCCTGGGCAACACGGTAAAACCCTGTCTGTACTAAAATACAAAAAAAATTAGCCAGGCATGGCGGTGTGCAACTGTAATCCCAG
CTACTCGGAAGGCTGAGACAGAAGAATCACTTGAACCCAGGAGGCGGAGGTTGCAGTGAGCCGAGATGGTGCCATTGCACTCCAGCCTG
AGTGACAGAGCAAGACTCCATCTCAAATAAGAAAGAAAGAAAGAAACAGAGAGAGAGAGAGAAAGAAAGAGAAAGAAAGAAAGAAAGAA
AGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGRAAGRAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAGAGAAAAGAA
GAAAGAGAAAGAAAGAAAAGAAAAAGAGAAAGAAAGAGTTGAGAAAGAAAATAATTTTTTATTCCATTTCTGTCCCCTACTCTACTCCA
CAGATTGAACGGTTTTTCAGGAAGATATATCAATTTCTATTTCCTTTCCATGAAGATAATGAAAAAGATCTCCCCATCAGTGAAAAGCT
CATTGAGGAAGATGCACAATTGACCCAAATGGAGGATGTGTYMAGCCAGTTGACTGTGGATGTGAATTCTCTCTTTAACAGGAGTTTTA
ACGTCTTCAGACAGATGCAGCAAGAGTTTGACCAGACTTTTCAATCACATTTCATATCAGATACAGACCTAACTGAGCCTTACTTTTTT
CCAGCTTTCTCTAAAGAGCCGATGACAAAAGCAGATCTTGAGCAATGTTGGGACATTCCCAACTTCTTCCAGCTGTTTTGTAATTTCAG
TGTCTCTATTTATGAAAGTGTCAGTGAAACAATTACTAAGATGCTGAAGGCAATAGAAGATTTACCAAAACAAGACAAAGGCAAGTATT
AAAAGATTACTTTTACTTAGAGGTTTACACTAAAGTCAAGTTTTGTTTAGCTTCAGAAATGGTAGACATTTCTGAGTCACATTGTATAG
CGTTTCTTGAAGAGACAATTTATGGAAAATGTTTCAGAGCCTCTTAAAAGAAGCTTTGAAGTCTGCTAAACACTATCCCTCTTCCATCA

FIG. 3K

TCGTTGAGAACTGAACTCTTTCTAGAGCAAATTTTCAAAGCAGAAAGAAAAAATGCTAATAGGTTGAGAACTTGAAAAAAAAAAACCAG
TTCCCTCATTTATTATTTCTTTATTTATTTTATTTTGTGACGGAGTCTCACTCTGCCACCCAGCCTGGAGTACAGTGGTGTGATCTTGG
CTCACTGCAACCTCTGCCTCCCAGGTTCAAGCAATTCTCCTGCCTCAGCCTCCCAAGTAGCTGGGACTACAGTTGTGCACCACCACGCC
CAGCTAATTTTTTTGTATTTTTAGTAGAGACGGGGGTGTCAGTATCTTGGCCAAGCTGGTCTCAAACTCCCGACCTCAGGTGATCCACC
CGCCTTGGCCTCCCAAAGTGCTGGGATTGCAGGCGTGAGCCACCATGCATGGCCATTTCCCTCATTTATTAAAGCTCATGTAGATGCTC
AGCTCTATTCTGCTAAAGCATCAGAGAGCTTCTTTAAAATTGATCTGGAATCCTCAACTCCCAGTTTGAGAAGCCCACTCTCACATATA
ACCAGAGCAATTTAGTGCCCTCCTCTGAATCACTACAATCATTCCTTAAATCATAAAATGTATGCATAAAACCACAAAAAATGCTCATA
AACCCCAAACTACAGAAATATTAGATAAGAATTGCCTTCTACCAACACTAATCATGCCTCATGGCATCCATGTTGGAGACACAATGCTG
CTTTATGTTTTAAGGCGGCAGATATCTTCTGTGGGCTTCTATGGAGTAAGTTAGATACCGCATTCGAGAATGAGAATTGCCACGAGGGT
CAAGTGTAGGATCTGCATTTCCTTTGTCACTGTATTGACCCCTAAGCCAGGTTGAAGGCTGCTCCCCTCTGAGATGAAAAATAAAATGG
GCTCCTTCTATCTATTTTTCTTTTTCTTTTTTCTTTTTTTTTTTTTTTGAGATGGAGTGTTGCTCTGTTGTCCAGGCTGTATTGGTGTG
ATCTCGGCTCACTGCAACCTCTGCCTCCTGGGTTCAATCAATTCTCCTGCCTCAGCTTCCCGAGTAGTGGGGATTACAGGTGCCCGCCA
CCACGCCTGGCTAATTTTTGTATTTTTAGTAGAGACAGGGGTTTCACCATGTTGGCCAGGCTGGTCTCGAACTCCTGACCTCAAGTGAT
CTGCTCACCTTGGCTTCCCAAAGTGCTGGAATTACAAGCATGAGCCACCACACCCAGCCAGCCACCACACCCAGCCAGCCACCACTCCT
GACCCTATCTGACTATTTTTCAATTATATTAGCTGTAGCTGGCAACATCTGAATCAGATTCTCAAAATCGCCATGACATTACATAACTG
GCCTCTACATAGGAGAGGTTTACCTTTCAGAAACTGAAGCTAGGAAACAGTGCATTACATCCTTCAGGTGCCATCGTTCCATGAACAGA
GAACAGCCATCATTACTGGAATTGTTGGGTTCTATTTCAGAGTCCAGTGGACTTTTTTTATAAGTCAATTATTTGGTCTGGTAGTCCAT
TCTGAGGTTGCAAATTCATCAAATATTCAGGATAAACACCAGGCGAGTAGACTAAATCTATCCAGGCTGGGTGGTATTAAGTGATTTTA
GCCTGACTGTTTACATGGATATCAACTGTCTTGGAATAACACTGAGAATATGTTCATTAGAACAAAAGGGCTCCTCCCCTCCATGTTGT
GTAGCAGCCTTACACAAGCATTGGTTACATTCCCATGTGCACAGGACTGTCAGTAGTGATTCAGACATGCCACAATCTAGATAATTTTT
CAACCACTGTAACCCCCTCCCACACACCAGCTACGAACATAGGTTTCCACTGTCTGCCACCATTGCCTTCTCATTCACACAGCTGGGGG
CCAGCCCTACTCTCAGCTGCCTCACACGCACCCTCCCCAGCCCCTCTGCGCCACTTCCATCTCAGTGATGACCTGGAAAGCCAAGGTCC
CCTGTGAATGCAAATAGTAAAGACAAAAACAAAATAGCAACCAAAAAAGTCTGTGTTACACTATTGTACTCTTCTTTCTCCAGTATCCC
TCCCCTAGCCAGACAGTACACAGAAGCTACCGCAGAGGAGACACTGTCTTCCCAGATGAGCAAATGTGGACTGTTTATCAAGAATAGTC
AGGCAGGCGCTCTACAGCACTTGAATGTGGTTTCCATCACTTTTCTGGACAGGTAGTTGGTGAGGAATAAGCCTACTGCCCCTAGAAAA
TCTGCCTAATGACTTGACACTTTGAGTTTTGCCCCTTGTGGTAGGCAAAATAATGACTGCCCACAATATCCCCACCCTAATCCCCAGAA
CCTGTGAATTTATGTTATGCGGCAAAGGGAAAGTAAGGATGCAGATGGAAATCAATTTGTTAATCAACTGACTTTATTTTTATTTATTT
ATTTTTTGAGACAGAGTCTTGCTCTGTCACCCAGGCTGGAGTGCAGTGGAATGATCTTGGCTCACTGCAACCTCCACCTCCGGGGTTTA
AGTGATTCTCCTGCCTCAGCCTCCCGAGTAGCTGTGATTACAGGCACTCACCACCATACCTGGCTAATTTTTGTATTTTGAGTAGAGGC
AGGGTTTCGCCATGTTGTCCAGGCTGGTCTCGAACTTCTGACCTCAAATGATCCGCCCACCTCGGCCTCCCAAAGTGCTGGGATTACAG
GCATGAGCCATCATGCCCGGCCTCAACTGATTTTAAAATAGAGAGAGTATGCTGGATTATCCAGATGGATTCAATGTAATCACAGGGTC
CTTAAAAGTGGAAGAAGGAGGCAGAAAAGAATTAATAGTAGCAGCCACAAGAGAAGGACTTGGCTCGACTTTGACGACCTTGAAGACAG
AGGAAGGGGCCAGGAGCCGAGTAATGTAGGTGGCCTCGAGGAACTGGAAATGGTATAGAAATGAATTCTCCTCTAGAGCCTCCGCAAAA
AACTAGCCCTACTGACATCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGAGACAGAGTCTCGCTCTGTCTTCAGGCTGGAGTGCAGT
GGTGCGATCTTGGCTCAGTACAACCTCCGCCTCCTAGGTTCAAGCGATTCTTCTGCCTCAGCCACCTGAGTAGCTGGGACTACAGGCAC
GTGCCACCACGCCCAGCTAATTTTTGCATTTTTTTTTTTTTTGAGACAGATGACATCTTGATTTTAGCCTAGGGAGACCCACTTCAGACT
TCTGACCTAAAAGACCAAACAATAATGAATTTGTGCTGTTTCAAGCCACTGAATCTGTGGTAGCTGTAGCAGAGCTAATAATAATAGTA
ACTGACCAACATTTACTGAGCAAGTTCCGTGTGGCAACCTTCATGGATGGGCCTTATTGGTCATGATTGTTTAAAGGGCCAAAATTAGA
AAAATAGCTAACACTGAATTATGAACACCAGGAAAAGGAGAGCGGAAATAAAAAGAATCAGAAATATCTTGATAATTAATGCTATTTTT
GTTGAGTATAGGTTCATTTTGTTCTCATATTTCTTTCCTACCTTGGTCTTTCTGGACCTCAGTTCCTGAATCTGTTGAAAGCGAATAGG
TCCAGGAAAGTAGCTCTTGGAATTATCTTCATTTGCCTTATGAATCCCTGGAAGGAACAGATGAGATTGAGTTCTACTGTAGCTTGACC
CGTGCGGGGGCCGGGAGACCTGGTTCTAATGCTGCCTTAGAGAGTGTTAGTTAACATTAATTTTCGCGTGGGAGAAACAGACAGGCAGG
TGGGAGAGTAGATGATTTAGCTCAGTGACTGCACTGGAAGTAGCTCCCTGGAAGGGTTCTGAGGTTCTGTCAAGGCTAGACTAAGCGAG
GTGATGGATTGTGCTGTGGCTGCAGGATGGGGAATTAGTGTCATATGGGCCTAGAATTTGTCATCCTTGGTGTACATACCAGGTATTAA
TCTAGATGCTAGAGATAAAATGATGATTATGACACAGCCTCTGACTTCCAGGAGCTCAGTCCAGAGAAAGGAAAACAGATTAGTGAACA
ATTACATCACCATATTGTGGGCTAAAATGGCAGAAGAAGGTATGGAAGAATGACAAGATTAAAATGGCAAGACCAAGTCCCTTCCCTCAA
GAGGCTTACAGTCTAATGGAAAAGATAAGAAAGCAAACACTACATAAAGCAGGAATTAATTCTACACTGGAAATTCTCACAGGGGGCTA

FIG. 3L

TACAGGGCAAAGAAGAGGGTCCAGGAAAGCAGCTGGGAGAAACTGACTTTCTGGTCACCAAAGGGGATGGGTGCCTTACATGCCATTCT
ATCAAACAGTGCTTCACTGTTTTTAAACTATGGACTTTGCAATTTATCTCAAAATAAAACGTTTCATTTTTAAATGCTGAGGATTTAAT
ATGACAGAAAATCATCAGGTTGTAAATTAGTAATACATGTTTCCTAATGTCAAACACTCTATTGGGAACCGCCAATTTTCTGTTGGATA
GACTTCTCTTTTACACATTTTTATATGGATTGTTAATTCTCCTAGGGGAAAAAACTTCTCAAAACTTGATTGGCTTTAGATATTTTCCT
AAATCTTTGACCCCCTGTTCATAACAGTATATGCATCTCCACACACACATACTCGCACACATATGTGTGTATATATATGTGTGTGTGTG
TGTGTGTGTGTATATACATATATATGAGAAATGCAAAAAAAGAATAGTAATAAAATAACCACCTATCACCCACTTTAAGAAACAGACAT
TTCTAATATCTTTGAAACTTCTTCCCAATTATAGCTTTAAAAATTAATTATTAAAGAGTTTTTTAAAATACAGAAAAGTCCAAGAGAAA
AAGTGGTTCACAATCACCTATTTACTTAATCCTATTGACATCAGAAATACTAATGATATAAGACAAATGATTTTTAAAGTAATCAAATA
TATAAAAGAACAAAATAAATGAAAGCTGCCCTCTCCTACCTTATCAACTCCCTCTTCTAAAAGATAGTTATTAATAATTCTTCATGACT
CCTCCTAGAAAATAAAATTACATGCATTAATATATGTGTGTATATACTACTAATAAATTTCTAGTAATGAGATTCTTGGATTCAAGAGT
GTGCAATTTTTAATAGCTGTTCAGTTGTCCCAGGAAATTATTGCACCAACGTGCATTTCTGTGTCTAAATATAGGAAAAAGGCCAGGG
GCGGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGTGGATCATTTGAGGTCAGGAGTTCAAGAAACCGGCCTGGAC
AACATGGCGCAACCCCATCTCTACTAAAAGTACAAAGATTAGCTGGGCTTGGTGGCTCTCACCTGTAATCCCAGCTACTTGGGAGCCTG
AGGCAGGAGAATCACTTGAACCCGGGAGGCAGAGGTTGCAGTGAGCCAAGATCCCGCCACTGCACTTTAGCCTGGGCAACAAGCAAGAC
TCTGTCTCAAAAATAAATAAATTAAATACATACATACATATAGGAAAAAGATTTTGAAAGCACTGGTAAGAAAAAGCTGCGGCATTGTC
TCCACTTCTTCAAAGTGCAAACTCTTATGACACTAACGTGTAAATGTTATGTTCCCTGTAGCTCCTGACCACGGAGGCCTGATTTCAAA
GATGTTACCTGGGCAGGACAGAGGACTGTGTGGGGAACTTGACCAGAATTTGTCAAGATGTTTCAAATTTCATGAAAAATGCCAAAAAT
GTCAGGCTCACCTATCTGAAGGTAAATAATTGCTATTTTGTTTTTTATTCTACTTTAAGTTCTCAGGTACATTTTGTTATAAAGTTTCG
GTGCCACAAAAGAAATAGCACTCGAATATAAAATTTTCTTTTTAATTCTCAGCAAGGAAAGTTACTTCTATAGAAGGGTGCGCCCTTAC
AGATGGAGCAATGGTGAGCGTGCACTTGCCAAGGGAGGGGAAGGGGTTCTTAACCCTGACAATGCACGTGGCCCCTGCTGCTGTGTGGT
TCCCCTATTGGCTAGGGTTAGACCGCACAGGCTAGACTAATTCCCATTGGCTAATTTAAAGAGAGTGACGAGGTGAGTGGTCTGGAGGG
AAAAATGGTTATGACAGAGCATGTAATCGGAATGAATCAGGGCGGAGCGTGTAATCGGAATGAATCAGGGCGGAGCATGTAATCGGAAT
GAATCAGGGTGGAGCGTGTAATCGAAAAAGGTTGCTTTACGAGGAAATTAAGTTTAAAAGTAGAAGGCAAAGAATTGAACATACTGACA
TACTGATTCTTTGGAAAGAAATTTAGAACTCACATCTAACAATTTTTTAGGGTTTCTTTAGTATTCTGGACAGAGGACAAAATCTCATT
CTCACAAGCATAGTGGATTCATTTGCTTTCCTCCAAGCACTTTTTTGCAGGCTCATTTCCATCTGGGGGCGTTCAATGTAGGTTTATAA
ACTGGTGTTTTGTTTGTTTGTTTTATGAGACAGAGTCTTGCTCTGTTGCCCAGGCTGGNGTGGCACAATCTCGGCTCACTGCAACCTCC
ACCTCTCGGGTTCAAGCAATTCTCCTGCCTCAGCCTGCCAAGTAGCTGGGATTACAGGCATGTGCCACCACGCCCGGCTAATTTTTTTT
GTATTTTTAGTAGGGACGAGGGTTTCACCATATTGGCCAGGCTGGTCTCGAACTCCTGACCTTGTGATCCGCCCACCTCGGCCTCCCAA
AGTGCTGGGATTACAGGCATGAACCACCGTGCCTGGCCTGGTTTATAAACTTTTATTATTCCAAAGTATGTCATTCTTTCACTTTCTTT
AATTCCCTAATTGTTCTTGTGATTTTTTTTATGATTAATGACCAAACACTATTGTGTGCAAAAGAAAAACCTTGAGCAAATTAGCGCAA
CTCCTTCCTTCTTACCGCAAGCAAAAAGAACCCCTGCCCCCAACCATGAAAGAAACCTTTCATTCTGTAAATCAGTGTTTAGACAAGTG
AAATATTTTTTTGAAAGTGGCATTGGCTCTTTCCCATTGGTGGGTTAATGAACTAATTAGCATTTAAATAGGGAAAGTGGCTTCTCCTC
CCAAGCCCCAGGAATCCTTTTCCCTCCCTTTCTAGTTCCTTCCCCAGGAAGGAAATCATTCTCCCTTTCCTCCATCCCTCCCCTCATTC
CCTTTCCCTTCTCCAGACTAAAGTCACTCCTCCAACCCCACCAGGGCCAAATTACAACTTTTCTTACATAAAACAAGAGCTTTTGATTC
CTATGCTTCTGCATTTTATCTCACTAAAGCCCTAAGGGAAGGAAATTTTCAAAGTGTGACTAATGGCTTACAGTAGGAAATTGGAAGAT
ACAGAAGGGACAGAAATCAACATGTCAGTAAATTCTACAACACTAGCTAGAGATTTGGGGCAAGTCATTTATGCTGTCTAGGCCTCAGT
TGAGTAATTTGTAAATAAAGGACCCAAGATAATCTTTGGGTTCTAACAAAATTCTTCTGTAAAACAGTGGTCCCCAGCCTTCTGGCACC
AGGGACTAGATTCCTGGAAGACAATTTTTCCAAAGATGGTGGGGCAGGGGGCACGTTTGGGGATGATCATCAGGCATTATTCTCCTAAG
GAGCGCTCAACCTAGACCCTTTGCATGCACAGTTCACAATAGGGTTTGTGCTCCCGTGAGAATGGAATGCCTCCGCTGATCTGACAGCA
GGCGGGGCTCAGGCAGTCATGCTTGCTCACCTGCCGCTCACCTCCTGCTGTACAGCTCCGTTCCTAAGAGGCTACAGGCTGATATGGGT
CCGTGGCCCAGGGGTTGGGGACCCCTGCTATAAAGGAAGTTCAGAAAAATCAGATTATAATTCTGATTTTTATAAATCAGAATTTATAA
AATTCAGATTATAATTTACTACCAAGTAATAGCTCTTTTGCCCTTAACTTCCCACAGTGAAGACCACTGGAGTAATTTATATCAACGCA
AAGAACAAAAAGCATGGTCAGTGGAAACTCCTGCCCCTCCCTTGGCTTTCTCTCCTCAATCTAACAGTGAGCAAGTTGCAACAAATCGC
GCCGTTCAGAGAAAAGGGAGGATGGAATTGTTACAACCGTTTCTGTCGCCCAGGCTGGAGTGCAGTGGCGCGATCTTCGCTCACTGAAA
CCTCTACCTCCTGAGTTCAAGCGATTCTGCTGCCTCAGCCTCCTGAGTAGCTGGGATTACAGGCACGCGCCACCATACCTGGCTGATTT
TTGTATTTTTAGTAGAGATGGGGTTTCACCATATTGGCCAGGCTGGTCTCGAACTCCTGACCTCGKGATCCTCCCACCTCAGCCTCCCA
AAGCGCTGGGATTACAGGTGTGAGCCATCGCGGCTGGCCAACAAATTGTTACAATGTTAAACAACATAATATCCTAAACATATTGGCTT

FIG. 3M

TTAAAGTATCATTAGATACACCACAATACTAATAAAGGTTACCTTTGGGTTTTAAGATTAAAGATGATTTTTAAAAATACTTCTTTCTG
TATTTTCCAAACTCTTAACCATAAACATAAGATATTCCTTGACTTAGGATAGGATTATGTCACAACCCATCATAAGTTTGAAAAATCAT
AAGTTGAACCATTGTAAATTGGGGACCATATGTACATGTATGCATATATGATATTAAAAATTATTAGACGTCTTTAAAATTTGACTTTT
TAACATATTACTTTTATTTAATCACCTTGCTCAAGGAGCCTGTAAATTACATATTAATATTCTCCATTATGAAATAAGTCTTTCCATTG
TGCAAATTAATGCATTGCAGAGGTTCTAAACATCTATATGCTTTGCAACTCGAAAGGAGTAAGTTTCCCTTTCTAATTTTTTTATTCAA
TTAAATAAAAAAATGAGTTTAATAGAGTCTATTAAATTAGATCATTATTCGGAGTGGTTAGTAAACCTGTTTAGAGTCGACAACACTCC
CTTTCTCTCTTTTTTTTTTTTTTTTTTTTTGTGCCAGAGTCTCGCTCTGTCGCCGAGGCTGGAGTGCAATGGCACGATCTCGGCTCACT
GCAACCTCCACTTCCCAGGTTCAAGTGATTCTCCTGCCTCAGCCTCTCGAGTAGCTGGGATTACAGGCAACCGCCACCATGCCCAGCTA
CTTTGTTGTATTTTTAGTAGAGATGGGGTTTCACCATGTTGGTTAGGCTGGTGGCGAACTCCTGACCTCAAGTGATTTGCCTGCCTCTG
CCTCCCAAAGTGCTGGGATTACAGGCGTGAGCCACCATGCCCAGCCCCTTTCTCCTTTTTAAATATCACCAGCCTGGGTTCTTTGTTCT
TTTTGTTTTGTTTYGTTTTTGTTTTTGTTTTTTTGAGACGGAGTCTTGCTCCGTNGCCCAGGCTGGAGGGCAGTGGCACAATCTTGGC
TCAGTGCAACCTCCGCCTTCTGGGTTCATGCCATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGGACTACAGGCGCCCGCCACCATGCCC
GGCTAAATTTTTGTATTTTTAGTAGAGACGGGGTTTCACCGTGTTAGCCAGGATGGTCTCGATCTCCTGACCTTGTGATCCACCTGCCT
CGGCCTCCCAAAGTCCTGGGATTACAGGCTTGAGCCACCATCCCTGGCCTCCAGCCTGGGTTCTTATTGACACTGAATTCTCAAGTTAG
TTGGGCTAGTGAGGAAGTCAGGTTACACGGGCCACAGAACAAGAACAAGGATTGTTCTTTCTCTCTCTCTTCCACTTCATTCTCTGTCA
GCCTCTCCCGACCTCAGTAGTTGGTCTTTTCTCCCCCTTCTTTTGAAAGCAGAGTCCATTATACAAATGGACTTGTTTACTTCTCCACA
TCCCTCTTGTGCAAATTTTCTGCCATGGACACCTCTACCCCACCTTAGAATGTATATTAGACAATTTTGACATCTAGAATGTCTTGTTG
GGCAGAAAAGCGTTTGGAAAGCGTTGCTCCAGGTAGCTCTGATTACAAACTGGACCTTTTCGCGGGGTTACCTAGAGCAGTTGAGAGTG
CTCTTTCTCCTGGCCAGGTGCAGTTGCTCATGGCTGTAATCCCAGCACTCTGGAAGGCCGAGGCGGGCGGATCACCTGCGGTCAGGAGT
TTGAGACCAGCCTGGCCAACATGGCGAAACCCCGTTCTACTAAAAATACAAAAATTAGCCAGATATGGTGGTATGAACCTGTAATCCCA
GCTACTCAGGAGGCTGAGGCAAGAGAATTGCTTGAACCTGGGAGGCAGAGGTTGCAGTGAGCTGAGATCAAGCCTCCAGCCTGGGCCTC
AGAGCGAGACTCTGTCTTGAAAAAATAATAATAATAATAAACAGATAAATAAAATTTAAAAAAATAAAAAGGAGTGCTCTCTCTCCTG
AACTGCTGACTCGAGGACTCTCTCAGCCTGTTTTATCATTTGGAAGAGGAAATAATATATCTGCTTCGTACACATCTTTAGAAGTTTAA
ATAAAATGTCTGAAATATCAATGATTCTCATTATTCAAATATTTGTTTTTTAAGTCACAGTTGCAAGGTTATATACAGAAGCATAGGTT
TTTATAACAGAAAAATAGACACTTAATATACTGACCTCTTACAAAAATAGTCCTGCTCAAGCATCCCATCTATGTATCATTAMCATCTA
TTTCTTTCTACCCAGCTAAAATAGTTTATTAATAATCCTTGAATGTCACAAGTNGAATACAGAATAAATCAGATAATACATTAAAATGC
ACCTGATAATCAATATGCACCAGATAATGGACACAGTATACATCAGATAATACAGTACAAATTCAATGAAAGTTTAGTGTTGCAAAGGT
AAAATGTAAAGAATGTCCTAATGTGCTCCCATGCTGCTTAAAACTGTTATTATAAATTGCTTTTTATTATAAATATATAAAGAATGATG
TAATAGGCCAGCCATGGTGGCTCATCCCTGTAATTCCAGGTCTTTGGGAGGCTGAGGCAGGTGAATCACTTGAGGTTAGGAGTTTGAGA
CCAGCCTGGCCAACATGGTGAAACCCCGTCTCTACTAAAAATATAAAAATTAGCCAGGTGTGGTGGTACGCACCTGTAGTCTCAGCTAC
TCCGGAGGCTGAGGCAGGAGAATCGCTTGAAACCAGAAGCCGGAGGTTGCAGTGGGTCAAGATCAAGCAACTGCACTCCAGCCTAGGTG
ACAGAGCGAGACTTTGTCTCAGGAAAAAAAAAAAAATTCTCAGTCACCTAGATTGAGAAATAGAACATTACCAAAACAGATAAAGCCCCA
CTGTGTTCCCATCCACATCACATTCACTTTATCTCCTCAAAAGGAAAGTGCTATTTTGAATTTAGTATTAATTATTTCCTTGCATTTCT
TCCTACTCATATCATGTGCCTATATACATATAATATATACAAATGCCGATATCATACATAGCAATGTTTTACATTTCGATTTTTGCATT
GTCAATGTAGAATTTTTAAACTTAAAAACATGCTTCATACAGCCGGGTGTGGTGGCTCATGCCTGTAATCCCAGCATTTTGGGAGGCCA
AGGCAGGCGGATCGACGAGGTCAGGAGTTCGAGACCAGCCTGACCAACATGGTGAAACCCCATCTCTATTAAAAATACAAAAAAAAATA
TTAGCTGGTCATGGTGGCGCGTGCCTGTAATCCCAGCTACTCAGGAGGCTGAGGCAGGAGAATTGTTTGAACCCAGGAGGCAGAGGTTG
CAGTGAGCCGAGATCGCACCATTGCACTCCAGCCTGGGTGACAGAGCGAGACTCCATCTCAAAAAAAAAAAAAAAAAAGCTTCATACAAA
CATGAAACGGGCACATGTCTGGCTGGGTGCGGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCCAAGGCGGGCAATCACTTAAGG
CCAGGAGTTCGAGACCAGCCTGGTCAGCATGGTGAAACCCCGTCTCTACTAAAACTACAAAAATTAGCCAGGCATGGTGGCATGCGCCT
GTAGTCCCAGCTACTCGGGAGGCTGAGGCACAAGTATCACTTGATCCCAGGAAGCAGAGGTTGCAGTGAGCCAAGATTGTGTCACTGCA
CTCCTGCCTGGGTAACAGAGTGATACTCTGTCTCAAACAAACAAACAAAAAAAACAAAGAAAAGAAAAAGAAAAAAGAAATGGGCACAT
GTCAAATGTTAATTTGACTATGTAACTTATTAATGAAGGAACCAGCAGGGTGTTAGAGCTGGGTCAAAGAAGTATAAGAGAGACTGGAG
TGCTTACAGTCAAGCAGAGACAGAATGCTGAAAGGTTATGAAATTAGATATGTTAGTTAATATTCGAAAGGGCAACTAAACTGTAAATC
TTGCCATTATCTTTTCTATCAGACCAAAATAATTTACATCTCTACTAGACAAACATTTGCCACTTTTCAATCCATAATCTATGGGTAAT
TTCATGGAGTCTGGCCCTAATCAACAGTAAATAGTAAAGCCAACAAAGGATCTCTTCCCTAGACCTTGAAGTGATCTTTGGGTGGACCC
CTTAGACAATAATTTAGTATGACATTGAGAGGACACGCAAGCCTGGGCAGCATAGTGAGACCCGGCCTCTACAAAAAAATTAAAAATTAG

FIG. 3N

CCGGGCATGGTGGTGTGAGCCTGTAGTCCTAGCTACTCAGGAGGCTAAGGTGGAAATACCACTTGAGCCCGGGAGTTCGAGGCTGTAGT

GAGCTATGATCATGCCATTGCACTCCAGCCTGGGTAACAGAGCGAGAACCTGTCTTGAAAAAAAGAAAAGAAAAAAGAAAAAGAAACA

AAAGGAAATGCAGCCATTTTTTTTTTGCCTTATTTCCAAGTTCTGGATAATTTTTCTTTTTTAACAATATAAATATTATCACTTATGTA

TTCTTTTGCAATATGGCTTTTCACTCAGTGTAGTTTGCAAGGGGTTAGCCATGTGAATGCATGCTGCTCTAGTTCATTAATTCACTGTT

GTATGTTGGTCTATGTAGGCATATCACAATWTATYCATTCCCTAGCTGAAGTACATTTGCTTTCAAGGTATTGCTATTATAAACAAATC

TCATACCTTTAATCAAATAATAATTTTGTCTCTTCAATCAGCTNTGATTTACTTTGTTCNAANACNAAGCACACAACTATAATTANAAT

TTCATTACTGATAAATATAAAATATTTTCCAAAACATCACAAATCTTTTNTNNTNCACTATTTACTATACACTTTNGGTCTNAATTTAA

AGCGGCTTCACTATATGTGGTTCTTTTCCTCTCTTCCCATACTAATTACTGGTACTGGACATATACATCCAAAATCAAATAGTARTGTC

CTTTTTAAGGGATAAATGGGATGTGATGTAGAAGGGGCATAGTAGGGACTTCATCTGTTTTGGCAAATTTTTTCTTAATATAGGTGGTA

GGCATGTGGAATTTATAACAAAAGTTCTGTCTCCAGCCCAGTTTCTGTTACATAAAACCATATAATTAACAGTTAAACTGGATCTGGTT

TGACACAGATGTAGACGATATTAATAATTACTCCAGAACAACAGGCATAACTAAAAACTACCACAGGCAAAAGGGGAAAATAGAGAATG

TAAGGGCTGGGACTTAAGCCCATGTTGCCCACCTCCAAGTTTCATGGACTTTTTCCTTCTCCACATTACTTTCTTCTCTGCTAGACTGT

CCTGATGTACCTGCTCTGCACACAGAATTAGACGAGGCGATCAGGTTGGTCAATGTATCCAATCAGCAGTATGGCCAGATTCTCCAGAT

GACCCGGAAGCACTTGGAGGACACCGCCTATCTGGTGGAGAAGATGAGAGGGCAATTTGGCTGGGTGTCTGAACTGGCAAACCAGGCCC

CAGAAACAGAGATCATCTTTAATTCAATACAGGTAAAGGAGAGACCCAAGAGCAGATACGGAAATGACACGTGCATACCTTGATTTCAC

TGTTAATTTACTTATGAATTGTGTCTGAATTTGAAAACAAGCTGTAGGAGGTATTCATATTTCCATTGTGATTGCCTTCAGGCTGACTT

GATTTAACGTAGTTCATGGTCTTTAGAAAACAAGAAAGTCCATAAAGAAAATCAATTTAAAACACAAAATACTTTCTAATCTAGAAATG

GCTATTTCTGCTTAGAGTTATAGGGCTATAACTGATAGAGGTAACCTTGAAGAAATATGGCCAATGTAGGTTTTAGGAGAGAAGACTTA

CAAATAAAGCAATTTGAGTTCAAAATTTGACTCTGAAACTTACCAGCTGAGTAAGCTTGGGAAAGTACCTCAACCATTCTAGGCCTCAG

TGTTCCACCTGTAAAATGGTAACAATCATAGCTATCTTAACGTGTACACCTATAAAGTGATTAGTATAGATTTCTTATACAAAACAAGA

GCTCTGTAAATTATAGCTCTTATTAGTTGCTGACACAATAAAGCCACTGAGTTATCTTGAGAATTAAACATTTATATGTTACTCGTCAC

ATAAAAATACATTGCCAGCTGGGCGCAGTGGCTTATGCCTGTAATCCCAGCACTTTGGGAGGCTGAGGTGGGTGGATCACTTGAGGTCA

GGAGTTTGAGACCAGCCTGGCTAATGTGGCGAAACCCCGTCTCTACCAAAAACATAAAAAATTAGCCAAGTGTGATGGCACACACTTGT

AATCCCAGCTACTCAGGAGGCTGAGGCAGGAGAATCACTTGAACCCGGAAGGCAGAGGTTGCAGTGAGCTGAGATCGTGCCACTGCACT

CCAGCCTGGGCGACAGAAGGAGACTCTGTCTCAAAAAAAACAAAAATAAARACATATTGCCATCTTAAATTCCACCTATACCATGACTC

CCAGATTCAGTCAATAACTTTTTGCATAACATGCAAGTGACTTTTCTTCCTAAGACATCCCCCCTCCAACACACACACATTACCTTAAT

CTACAAATGCGCCAGGCTAGTGATTCCTGATGAGGCTGGTTTTGAGGGTTCCCAAAAAGACTTGGATACAAAAATTACTGGGCAGAGCA

ATTGAAGATGCAATATTCTGTGTGTAGTATGTTAGGTTATGTTGGTGCCCTATCCAGATCCCTGGGGATCCCTTTTACCAGCTCCCACT

GGTGCTGGTGCTGCTGCTAACTGCTTATCTCTGAAACTTTCTCCCAAAGATTGCCCTTGGAGCACTTATGCCCCAGAGCTTCCTGCAGG

ATCAGGCTGAGGCTAACAGTCATCTGAAGCCATATCCTTGCTTAGCTTCTTTCACTTCTCTAGTTTGCTTTCCTCATCCCCTTAAAAGT

TGCACCTGAGAGCATTCTTTATAAACCACTTCTGTCAGAATCTCAGGCACTGCTTCTAGGAAATTAGACTTATGGCATTCTATAATCCA

GCATTTCCCTCTTTTTTCAAACTACAAAGCTGTGGATCATGCCTGATTTGAGAAATAAGTTTAGAAAGTCACAGCAAGCTCATTAAAAA

ACAAAATTAAAAACCATACAAAAAATAGAATAGGACAAAGTAGAAAATATTAGCATGCATTGCATTTCATAAGTCATATGCACATCATG

GAATTTCATTTCCATTTTGTATGTGTATATGTGTGTAAACATATATACACATATGTAGACATACGTGTGTGTTTTGAATCATGATGTCA

AGTGTATTCATTACTGCAGACCACAGTCAAAGGGTTTTGAAAGCCACTGTTCCAATCCCTGCCAGCTCTCTGATTCTATAACTCTATTA

GATTACACTTGAGGAAGGTAAAATAATTCAATATATTTGATCATCCTCGCATATATAGACTTTTAGTTTAACGAGGAAAAAGTCTTGTA

TTGAAGAATAAAACTTGAAGAAAAATTTTAGCAGTGCTTTCAACCTTTAGAAATCTACAGTCAATATTTAGTTGTTTTTACCATTGTCA

GTATTTTCTATTCTGTGCTTTGATTTACTTCCATTCTAGTGTCTCTTGAGTAACATAACAGATTTATCTAAAATTCTTTATGCTGATAA

CAAAGGCACTTCTATATAAAAACCTCCACATAAAATAAAATTATGGTTTTCAATTATACATTTTTATAACAATTATTACCACTTAAGAG

CATTTACTGGGTGTCAGGCAATGTTCTAAGACTTTTTCCATATATCAGATCATTTAATACCCTCAATGACCCTATAAGGGAAGTAGAAT

TCTTTCCCCAGTTTTTCAAATGAGGCACAGAGGAGGTTAAGCAACTTGTCTGAGCTCACACAGCTAGTAAATGGTAGAACTAGAATTCA

AACTCAAGCAGTATTTCTCTAGAATCAGTGAACGTAACCACTTTGCTAAACTGCCTGTGAAGTTACTTTTCTCAAAACAGCTCCTATTT

CACCATGTAAAGAAAAGTACAAACCCATAAAATAGCAAGTGCTGAAGAGAAGCCTTATGAAAGAAATATACAAATTCCAGCAAGTGAAA

ACGGTTGTGGTCCCTGGTTGTATAATAGTTACATGGGTGTTGACTTTACAATTATTTAAACCAAACATAAATACTTTATGCAGTTTTTA

TGTATGTTATACTCACAGAAAGAGAAGGGAAAAATTTTTAAATCATTCTCTTAAGGTTACATCAAGTTGCGTATCAGTTCAGTTCCATT

TAAATGATTCAAATCAAAGTCTGTGCATTTGAGAATTCATTAAGAGAGTAACATACATGTTATTCATTAAGAGTAACATAAATTTTGCA

TTGATTCTTGCCAAAATCACACCTACAACCATAAATTGTAAATTTCTAGGAAAACTCAGTACAAAACTTGGTGCAATGCAATAAAGTTT

FIG. 30

GTGGCACAGACAGTAATACTCAGCAAACATCCCACCTCCTCTCTCATATTTTCCAGCTCCCCTTGTGGTTAAACGTTGCCATGTGGCAA
GTTCTGGCCAGTGAAGCGTGAGCAAAACTGAAAAGGGTTCTTTGTAGATTGAGACAGTGAAGAGCCTATGTGTGCTCATCTATTCTCTT
TTTCTGCTGAGGGCACAAAGAAAGTCCTGAAATCATGTGCTACAGCTATGAGATAATGTGCCTTTGCCTACCAGGCTTCTCAGTGTTTA
CTGGTGTGGAGCCCTTGTAATGGACACATAACATGAACAAGAAATAAATCTTTGTTGCATGAAGCCCTAGGAATGCCAGGACTAATCTG
TTACCTCAGCACAAACCCAGGCCTATCCTGACTAAGGTGGTATTAAATTACTATTGAATGTGTATTGGGATTTAGTAAACTTCTACTGT
ATAATCCTTCTTCTGTAGGTAGTTCCAAGGATTCATGAAGGAAATATTTCCAAACAAGATGAAACAATGATGACAGACTTAAGCATTCT
GCCTTCCTCTAATTTCACACTCAAGATCCCTCTTGAAGAAAAGTGCTGAGAGTTCTAACTTCATTGGCTACGTAGTGGCAAAAGCTCTAC
AGCATTTTAAGGAACATTTTAAAACCTGGTAAGCAGAGTGCCTGGTTAGGAATGCCTTGTTGACAGGAATAGTTAATTCTCAAAAGGGA
AAAACAAAACTTGTTTCAAAATACCTGGAAAAACATGTTTAACCTCATTAATAAAGACATGAAAACAAACAAGATGGCATTTTCTGCCTA
TCAGATTTGCAAATTAAAAAAAAACCCAGGAAATCCTGATAGGAATGTGATGAAATGGGAATTCTCATATATCATGTATTGGTGGGAAC
ATAATTGGTTTTGCATTTTTGAAAGCTATTTGATTATGCATATGAAGAGCCATAAAATTTCCTTTTGATATAATAATTCCACTTCCGAA
ATCAATCCTAAGGRATAAATCTAAATTTGATGAAMAKTCTCCCTCCAAGATCTAGATTTGCAGCATTATTTAAATATTAAAAGTTGGCC
GGGCGCAGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCTGAGGCGGGCGGATCACGAGGTCAGGAGATTGAGACCATCCTGGAT
AACACGGAGAAACTGCGTCTCTACTAAAATAAAAAAAATTAGCCGGGCATGGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTG
AGGCAGGAGAATGGCGTGAACCCGGGAGGCAGARCTTGCAGTGAGCAGAGATCGCGCCACTGCACTCCAGCCTGGGCGACAGAGCAAGA
CTCTGTTTAAAAAAAAAAAAAAAAAAAAAAAATATATATATATATATATATATATATATATATATATATATATATGTTAAACATACTCTTAA
TGTGTAAAAACAAGAGAATGATTAAGTAKATTATGACTAAATACACTCAATACATTTTATGAAACGTTAAAAATATTCAAAAAAATTTAA
ATAATGACTTGCTAACTACTTTAACAAGAGCTTTATTATCAGCTAGTCTTGGAGGTAATAGTATTATCATGATTTTTCAGAAAAAGATC
CTGAGGCTCAGTGTCCAAGGTCCAATGAACTACTCAGGTCGGAGGTGGTAGAGCAGCATGTGGAGCCAGTTCTCTCTCCGACTCCATCA
TCACACTGCACGGCTTCCTGTTAAGATATTTGCTCAAAAAATGCGAGATATAAAAATCTGGGTAATATGATCAACCTTAAAGAATAATT
ACATTTTAAATTATTCATGAGACCTTGTTAGTAGGTCACCATCAATGTGTAATTAAGCCAGATGTGACAGGATTTGTTGCCTCTCCCTT
TACTTCTGAATTTTGGAGGCCTTTTTTTTTTTCTAGTTGTATCAGTCAGCCAACCAATATCTTTTTAGCATCTACTAAGTTTAGATACG
GGAACTGGTACTCTGAAAGAGAAAATGAGAAATTTGACAAGATCCTGTCCCCAAGGAGCTTCCTATCCAACAGGGGCACAAGACAGATA
GATAGACACACACACACACACACACACACACACACACACACACACACTATAAAGCAAGGCAAGATTTAGAGAGTGCACAGGAGTGGG
CTCTGGGAGTTCAGGGGAGGGTCGTTCACATTCTGGTAGGGAAGATACTTCTGAGCTCAGTATATTCCCTTTCTCACTGTCCTTCTATC
CCCTCTCTTCCTCTCCTCCTCTCTTTTCCTCTTTCTTCTCCCTCCTCCCACTCTGTCCTCTCCCTTTCTTTCCTTTTTTCTTTCTTTCT
TTTTTTTTTTGAGACAGAGTCTTGTTCTGTCACCCATACTGGAGTACAGTGGCACGATCTCGGCTCACTGCAACCTCGGCCTCCCAGGT
TCAAATGATTCTTGTGCCTCAGCCTCCTGAGTAGCTGGGATTACAGGCGCACACCACCATGCCTGGCTAATTTTTGTGTTTTTTAGTAG
AGACAGGGTTTCACCATGTTGGCCAGGCTGGTCTTGAACTCCTGACCTCAAGTAATCCACCCACCTTGGCCTCCCAAAGTGCTGGGATC
ACAGGCATGAGCCACCACACTGGCCTCCTCTCCCTTTCTTAAAAATACATCAATTAATTAAATATATAAATGTAGATACACACACAGGC
AGAATCAAAGTGTATAGGTTGGAGAGGAGACTGTTCCAAAAGGGGGGATGGCATGGGCAAATACGGCAAGAAAGAGTAGAGCATCTAGG
TACTGAGGGTGCTGGGAAGTCCTGCTAAAAATACGGCAAGAAAGAGTAGAGCATCTAGGTACTGAGGGTGCTGGGAAGTCCTGCTAAAG
TGGTCCCCTCCCACTGTGGGGCCTTTGAGTTTCCCTGTGCCAGGGTACCTGCCCTCTGTGAGTTTGAGTTCTTTCTTTGGTTGCAAGCA
ACCAAGACCAGCTCAGCTAAAAGAAATGGATGGATACCGACTCATGAGTCAGAGGGGAAGCTGGACGTCTATGCCCAGAGCCAGGCAGA
AACGGGTCAGGTCTAGAGTCTGGGAGGAGGAAACCGATGGACAGCTGCTTCAGGGCCCAGCGCTCAGGGTGAAGCAGCTGCAGTTGTTT
TTAGTCCTCAGATCACTCTGCTCAAGATGTGACTTGCCAGGAGGAATCTGGCTGGCCCAGCTGGGACATGTGTGTCTACCTCTAGACCA
GGAGAGAGGAGAGTCTTGGTTGACAGTCCCCATGTAGTACCCCTTTGTTTAGGTTACTGAGTCATCAACAGATCTCAGTTCAAATAGTC
ACTTCTTCAGGGGCAATATACCCTCTTCTACCCATAAACTAGGGGCAACATACCCTCTCTCCCCTTTCACACATGACCATAACACCATG
TAGCACTCAACTCTTGTAAGTTGACATTTACCCATGTCACTCTTTATGAACGTTCATCTCCATCCCGAGACCTACAGTCCATGAGGGTA
CCACCGTTCTAGGGTTTTTGCTCTTCTCTTTGTCAGTGGGGACTTAGGACTCTGCCTGGCACAGGGCAAACCCTCAATATTTGTTGAAT
AAATTAATTAATAAACACGTGTAAATGAATATCAGTAGACTACAACAAGAGTAACAGTAGGCGAAGGTGGAAGGCAAAGGTGGGAAGAG
GTCAGGGCTCTGAGTGCTGGGCTGTGGAGTCTGAGGTTCACTCTACAGCGCTGGTGAGACACGATAGGTTTTAGAGAAAGGAAGCCTCA
TGCTGGTGCCCCAGTGGGTACTGACTATGCATTTGTAGCCAAATCAAAGTATTTCCCATAAAGTCATCTATCTCTTCCCAGTTGTTGGG
ACTTCCAATGGCAATGGGAATTAAGATACTGAGTAATTGGGAGATCAAGCAAATTATTTACTAACAAGGCACACGAAGTGATTTTTCAC
AGGCAATGTTAATGTTTTTCTTTTTTATGTAGTTTTAAAATTCTAAAAGTAACAAAATCACAACTACCAAACATTTAGACGACAAAAAT
TATCCATAATCCCACCATCTTAACACAACCACTATTATCATTTGTTTTCCTTATTCACATTTTCTACCTATTTTCTTAGATTYCCAAGA
AATAGAATTACTTGTTTAGAGGTTATTAACATCTTATTGTTCTGGATATATATATATATATAGCTATATATAGCTAAATTTAATAACAG

FIG. 3P

CAATGTCTGCAGTACCACTTTCTCAAATGCTAACTGGCATTTCAATTTTTTGAGACAGTCTCTCTCTGTTGCCCAGGCAGGATTGCAGT
GGCATGATCTCGGCTCACGGCAACCTCCACCTCCCAGGTTCAAGCGACTCTCATGCCTCAGCCTCCCAAGTAGCTGGGATTACAGGTGT
GCACCACCACACTTGGCTAATTTTTGTATTTTTTAGTAGAGATGTGTTTTTACCATGTTGGCCGGGCTGGTCTCAAACTCCTGGCCTCA
AGTGATCCTTCCACCTCAGCCTCCCCAAGGGCTGGTATTACAGGCATGAGCCACTGCCTGGCCTGGCATTTCAATTTTTAAAATCTTCA
GTAATAAATGAAAATTTTTATCTTATTGTTATAATTTTTATGGTTTTTTATTATTCATGAGAATAAACATTTTCCAAGTTTGTTTACTG
ACTGAATTTCTTTTTTGTGCACCTTACTTGGTATCATGGATAAAATTTTGTCAATTTTCTGATTATATCAATGCATTCAGGGTCCCAAA
CCTGCCAAAGTTTAAAGAGAAAGATACTAAGGGAAAAACCAGGAAAAGATGGTAGAAAAGAATCACCCTGGCATTTTCAATCACGTAAA
CATTTGCTAGGTGCCCTAGCTGCAGGTATACAGCTCACTGAAACATGAATTCCAATTTTATAGGGTGAAATATATATTTAGAACCCTCT
TCTGGAACTTTCTTCTAGTTATCTAGCATCCTAAGTGCCTGGACGTTCCTGATTGGTTTGCAATGTGTTTTATTTCCCATCCCCAAGTT
TCATAGCTGCCGGCCCTGGGATCTACAGTCACAGGCTGTAACACAATATCTTGCACATCCTGAGTCTTTAATAAGCTTTTGTAGATGGG
CTCTTACCATCATCATCATCGTGAAAGGCAAATATACAAAATTTGTTGACTAATGTAATGAGTCATGAGTAACAGAAGTTTACTGACCA
AACACTACGTGCATGTAGAGTTCAGAATAAACACTTTATTATCACATCAGAGGAAAAGACCATCTTAGAGGCTCAACAACCCAGGAAAG
CTGTGACGATTTCTTCAAATTGTTAAGAATATCCATGCATATGGGTTTCACATTATTTTGCTACACACAGTACCAATTTTTCCAAAAGC
CAACAGCAGGTATTCTATTACCCATCCTGGACTTTTACTCCAAGAAAAAATACACTGAGTCTGTGAGTAATTTATTAGTATTTTGATCA
TTGCTGCTTTTTTTTTTTTTTTTAAGGTAAGAAGATCTAATGCATCCTATATCCAGTAAGTAGAATTATCTCTTCATCTGGGACCTGGAA
ATCCTGAAATAAAAAAGGATAATGCAATAAACACAGTTGCAGGAAAGTATGTTAGCTATATACTATGAAGTACTCTTAGTTTACTTATG
TTGAATGGCTTAGCTATTAATACTCAAATTGAGTTAAAATGAAAATTCCTCCTTAAAAAATCAAACGTAATATGTATTACATTTCATGG
TACATTAGTAGTTCTTTGTATATTGAATAAATACTAAATCACCTAGGTGTCTATGTTCTATCACATCTACAAACATGTCACTTCCTAAT
TAACAAAATGTTCTTCCTTTAGTTTGCTTTTGCACTTAAAATATATATAATTGACTTTTTTGGAAAAAAATCTAAGATTCATTGCTTTG
TTTTGTAAAGACCAATAGGTTCTGTATAGTCTTTTTTTAAATTGTGGTAAAATACACATGGCATTAATTTACCATTTTAACCATTTTAA
AGTGCACAATTTGTGGCATTAAGTACACTCACGTTGCTGTGCAACCATCACCACCGTCCATCTTCAGAACCTTTTTATCTTCCTAAACT
GAAACTCTGTACTCGTTAAGCACTCACTTCCCGTTTCCCCATCCCCCAGCCCGTAGCAACCACGACTGTACTTTCTATGAATTTGACTA
CTCTAGGTACTGCATGTAGGTGGAATCATACAGTATTTGTCTTTTGCTTCATTTTGTTTTGTTTTTTGTTTTCTAAGACAGGGTCTCAC
TCTGTCGCCCAGGCTGGAGTGCAGTGGTGCAATCACAGTGTCCTTTTGTGACTGGTTTATTTCACTTAGTGCCATGTTTTCAAGGTTCA
TCCATGTTGTTGCATGTCTCAGAACTTCCTTTTTTAGGCTAATATTCTTGCATGTATTTACCTAGTTTTGCTTATCCATTCAGCCATTG
ATGGACACTTGGGTTGCTTCCATCTTTTGGCTATTGTGAATAATGCTGTTTTGAACGTGGGTGTGCTACATAGTTACTTTTTAAAATTG
GCACAACAGCGCTGTCTTTTGACATACGTATTTTATGGAAAACACAAGATTTTCCTGGCTGACGCTCAACCTCATAATTTGGACCTTGG
TGCAACACAATAATAGGAGAGCTATGTGTCAGTATATATCACTAAGGATTACAATGAGAGTGTATACAGTCAGTATTACAAATTATAAA
AAGAAATGTAGGCCAGGCACGGTGCCTCACACCTGTAATCCCAGCACTTTGGGAGGCCAACGTGGGTGGATTACCTGAGGACAGGAGTT
CGAAACCAGCCTGGCCAACATGGTGAAAACCTGTATCTACTAAAAATACAAAAATTGGCCAGGTGTGGTGGCGCATGCCTGTAATCCCA
GCTACTCAGGAGGCTGAGATGGGAGAATTGCTTGAACCTGGGAGGCAGAGGTTGCACTGAGCCAAGATTGTGCCACTGTACTCCAGCCT
GGGCAACAGAGCGAGACTCTTTTTTAATAAATAAATAAATAAATAAATATATAAAAGAAACGTAATGAAAGAGAGAGAACTCTGAACTT
TTAAAGAACTTTTCACCCAGTCTTGATCTATCTGACAGAAAGGCTTGTCAGAGAAAGTTAGAGTTCAGAGGCAGCCAATTGAATATAAT
TAACTCCAAATGAAGATAAACCTTTTCTAAATCATACTGAAGGCTATAAAAAATGAGAATTATGTTATTTTTTTTTTGAGACAGGGTCT
TACTCTATTGCCCAGGCTGGAGTGCAGTGGCATGATCTGGGCTCACTGAAGCCTGACCTCCTTGGCTCAGGTGATCCTCCCACCTCAGC
CTCCTGAGTAGCTGGGACTACAGGTACTACCATGCCCGTCTATTTTTGTATTTTTTTAGTAGAGATGGGGTTTCTCCATGTTGTCCAGG
CTGGTCTCAAACTCCCAGGCTCAAGCAATCTGCCCGCCTCAGCCTCCAAAAGTGCTGTAATTACAGGCATGAGCCACTGCTCCTGGCAG
GGAACTAATAGAATCCTGGGTTCTTCGGTGTGCAATAAAYCTCAAATACAGCTATTCAACCATAGATTTTAAATATTTGTTAGTGAAGG
TGACAAAAAAAATAAGTGATTAAGAGAACCTATTTTCTATCCAATGAGCTATCAAAAGCTTATAGAGTGGAAAGAGAGTGGGGGAAGTGA
GGCTCAAAACAGCTAAATGGAAAGAAGATTTTGCATGCAGGCTGAACTGGATTTTCATCCTGGCTACTATATTCTCCAGATGTGTCACT
TTGGCCAAGATCCTTAATCTCAGTGTCATCTATAAGGTAATTAAAGTACACTAGTGCCCCACTAATCTGTGGTTTTGCTTTCCAAGCTT
TCAGTTACCCGAGATCAACTGCGGTTTTAAAATATTATGTGGAAAATTCCAGAAATACATAGTAAGTTTTCAATTGCATGCCATTAAAT
CTCATGCTGTCCCTGACCCCTTCCTCTCCGGAGGTGAATGCTCCCTTTGTCCAGTGGCTCCACGATGACTACATTCCCCAAATTGTTCT
CTTAGGAACCCTTTCTGTGTTCAAGGAACCCTTACTTTACTTAATTATGGCCCCAAAGCACAAGATAGGGATGCCGGCATACTGTTATA
ATTGTTCTATTTTATTATTAGTTATTGTTGTTCATCTCTACCTGTGACTAATTTATGAATTCAACTTTATCATAGGTATGTAGGTATAG
GAAAAAAACATGGTATGTATAAGGTTCAGTACTATCTGCAGTTTCAGACATCCCCTTGGGGTCTTGGAACATATCCCCCGTGGATAASG
GGAAACTACTGTAAAAGTTTGTSTTTTATAGAGTAGTTSTSAGAACTACATTAATCCATAATGTGTGSCTCATGATACTCATTGATAGA

FIG. 3Q

TGGTAGTAGCAACAATAAAAAATAATATTATCAAGTAACTGATTCATAATTGACTCTCAAAAACGTTAATTTTCTGCTTTCCTTTACCT
AAGTTTACCTACATGTTTGAATTTGTAAAGGGAAGGTTTTTCTAGACCAATAATTTTCAAATATTTTTGCTCTCATACTTCCTCAAAGG
AAACTGAAAAAGTTGCAACATACTTGCATGTCATTTTTCTATATAAGTTGAAAGAATAGCAAATTGTTATTTTCCCACGCATCGTAAAG
ATTAGCAGGTCATCCCTCTTTAAAATGTACCAAATGGAATCTAAATATCATCGCAATTTGACCCAGCATCATCCATTTAAACAAATATA
CAAGTTTTTCTTTAACAATGAGAAATTTTATCTCATTACATTTTCTCCCTAAACTCTTATTTCAATCTACATTCCTAAGAATTTTATCC
TAATGTAGTATATTTTTATGCTTAAATATCTTTTGTTGATCAACACAATTTTGATCATTTTTAAATTTTAAAAATTAAGAACATCCTGT
GACATCAAATTCTAGGTATGAAATATTTATTCTAGATTGGGTGATCATTATAATTATTTTTTGTACATAATTGATCAAAATAACATAAA
TATACTACAAATTTCTATGACTACTAAACATATAAAAGTAAAATTTTAAACAAATATATCTCTTAATGAGAAGGAAGAGCTTTTTATAC
TCCAATAAGTTAACGTATCCACTAATAATTATTATTTCTTCCTAGAACAAGACAGGATTAAGCATCATGACCGTCCCTATTGGGGGATG
TTTTTATAGATGCAAGCACTGTGGCACCTACTGGTATAAATGCACCTGCTGATTGGAATGTTCTTTCCCCAGATCTTCCCCTGCTGGTT
TCTTCCCAGTATTCAGGTCTCAGCTCAAATGTGACTTCCTCAATGAGGCCTCCTGGTGATCAGATCTAAAGCACCCTCTACACAATCAC
TGTTTAGTGCTATACCCATTAATTTACTATCATCACACTTGTCACTATCTGCAGATGTCTTGTTTGGTTACTTTTGTNGTGTTTGTCAC
TGCCAGAATATCAGTTCTATGAAGAAAAGGGCCTTGTCTATTTTGACACTTATAGANATGATGNAGGNACGACATACAAATGGCCAATG
GGCATATGGAAAAACGCTTGACTTCAAGAGTACTNATGGNTATNACCAACATTTATGGAGTAACTACTTTGAAAAGAACCATTCTGTCT
TTACTATCAAGCCAAGATACTCAAGGAAGGCAGCAGAAGTGGAAGCTCCATGTGGGCAGAGGAGCCTAGTCTTGAGATGTGATTTAGCT
GGTATTTGGGTGAAACAAATAAACCAGCCTCAAAATAACACAAGGGGCCGGGTGCAGTGGCTCACGCCTGTATCCCAGCACTTTGGGAG
GCTCGAGGCAGGCAGATTACTTCAGGTGAGGAGTTCGAGACCAGCCTGGCTAACATGGTGAACCTCCAT

FIG. 3R

7853-138

HKNG 1 mRNA expression in normal brain

Brain Regions	Gray Matter	White Matter	Neuron	Astrocytes	Oligodendrocytes
Frontal cortex(1)	+++	-	++	-	-
Motor cortex(2)	+++	-	++	-	-
Parietal cortex(3)	+++	-	++	-	-
Occipital cortex(4)	+++	-	++	-	-
Hippocampal formation(5)					
CA1	+++	-	++	-	-
CA2	+++	-	++	-	-
CA3	na	na	na	na	na
CA4	+++	-	++	-	-
Dentate gyrus	++	-	+	-	-
subiculum	+++	-	++	-	-
parahippocampal gyri	+++	-	++	-	-
Caudate/Putamen(6)	+/-	-	+/-	-	-
GPi/GPe/Putamen(7)					
GPi	+	-	+	-	-
GPe	+	-	+	-	-
Putamen	+/-	-	+/-	-	-
Amygdala(8)	++	-	+	-	-
Thalamus(9)medial	++	-	+	-	-
Substantia nigra level(10)					
SNc(substantia nigra pars compacta)	++	-	++	-	-
SNr(substantia nigra pars reticulata)	+	-	+	-	-
Red Nucleus	+	-	+	-	-
3rd cranial nerve nuclei	+	-	+	-	-
superior colliculus	+	-	+	-	-
Upper pons(11)					
Locus ceruleus	+	-	+	-	-
pontine nuclei	+++	-	++	-	-
Lower pons(12)					
locus ceruleus	+	-	+	-	-
pontine nuclei	+++	-	++	-	-
raphy nucleus(midline)	++	-	+	-	-
Medulla(13)					
inferior olivary nucleus	++	-	+	-	-
12th cranial nerve nuclei	+	-	+	-	-
nucleus ambiguus(multipolar lower motor neurons)	+	-	+	-	-
Cerebellum(14)					
Purkinje cells	++	-	++	-	-
Granular layer	+	-	+/-	-	-
Molecular layer	+	-	+	-	-
Temporal pole(15)	+++	-	++	-	-
Cingulate cortex(16)	+++	-	++	-	-
Anterior thalamus(17)					
Subthalamic nucleus	?	-	?	-	-
Ventral anterior N. (VA),Ventral lateral N.(VL)	++	-	++	-	-
Hippocampal formation(18)					
CA1	na	na	na	na	na
CA2	na	na	na	na	na
CA3	+++	-	++	-	-
CA4	+++	-	++	-	-
subiculum	+++	-	++	-	-
parahippocampal gyri	+++	-	++	-	-
cervical cord (rostral postion)				-	-
anterior motor nuclei	++	-	+	-	-
sensory nuclei group	++	-	+	-	-

FIG. 4

pedigree	Affected Individuals	Phenotype	a.a. change	exon	comment	nt change	nt position
30124	3010189	scz	R331T	8	3 of 4 affected individuals	AGA -> ACT	51,641 51,642
	3010185	scz					
	3010184	scz					
30105	3010027	scz	I23T	3	the only affected individual	ATT -> ACT	35,044
31102	3110017	major depr	E202K	7	all three affected individual (also seen once in Costa Rica)	GAA -> AAA	45,487
	3110014	scz					
	3110003	scz					
30120	3010155	scz	E202K	7	one of the affected individuals	GAA -> AAA	45,487
30126	3010203	scz	intronic	10	3 of 4 affected individuals	insertion: GAATGCCTGGTTAG 21 base pairs 3' of exon 10	after 63,417
	3010210	scz					
	3010204	scz					
30140	3011486	scz	intronic	6	one of the two affected individuals	A -> T (24bp downstream of exon 6)	43,450
32301	3210041	scz			two of the three affected individuals		
	3210051	scz					

FIG. 5A

pedigree	Affected Individuals	Phenotype	a.a. change	exon	comment	nt change	nt position
30120	3010155	scz	L34L	4	one of the two affected individuals	CTC -> CTA	36,307
32200	3210104	scz	L34L	4	both affected individuals	CTC -> CTA	36,307
	3210009	scz					
31109	3110013	scz	I23T	3	one of the two affected individuals	ATT -> ACT	35,044

FIG. 5B

a.a. change	exon	nt change	position
non-coding 5'-UTR	1	G->C (35 bp upstream from 3' endo of exon 1)	15,385
L42L (silent)	4	CTG -> CTA	36,331
V123G	6	GTT -> GGT	43,184
non-coding (intronic)	6	A -> T (24 bp downstream from exon 6)	43,350
V30I	7	GTC -> ATC	45,571

FIG. 5C

AGTTGCGTCCCTCTCTGTTGCCAGGCTGGAGTTCAGTGGCATGTTCATAGCTC
ACTGAAGCCTCAAATTCNTGGGTTCAAGTGACCCTCCTACCTCAGCCCCATGA
GGACCTGGGACTACAGTTCCCTCCCTTTGGAACGCAGCGTGGGCACCTGCAA
CGCAGAGACCACTGTATCTCCGGTGCAGAATGTAATGAGTGCCTGATACATT
TGCCGAATAAACTATTCCAAGGGTTGAACTTGCTGGAAGCAANAGAAGCACT
ATTCTGGTAACAGCGGGAACATGAAGCCGCCACTCTTGGTGTTTATTGTGTGT
CTGCTGTGGTTGAAAGACAGTCACTGCGCACCCACTTGGAAGGACAAAACTG
CTATCAGTGAAAACCTGAAGAGTTTTTCTGA

FIG. 6A

AGTTGCGTCCCTCTCTGTTGCCAGGCTGGAGTTCAGTGGCATGTTCTTAGCTC
ACTGAAGCCTCAAATTCCTGGGTTCAAGTGACCCTCCCACCTCAGCCCCATGA
GGACCTGGGACTACAGATGGAGTCTTGCTCTCGTTGCCCAGACTGGAGTGCA
CTGCTGCGATCTCAGCTCACTGCAACCTCTACCTCCCAGGTTCAAGCGATTCT
CCTGCCTCAGCCTCTCGAGTGGCTGGGACTATAGTAACAGCGGGAACATGAA
GCCGCCACTCTTGGTGTTTATTGTGTGTCCGCTGTGGTTGAAAGACAGTCACT
GCGCACCCACTTGGAAGGACAAAACTGCTATCAGTGAAAACCTGAAGAGTTT
TTCT

FIG. 6B

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CTTGGAGTCAACTGAGTGTGGACTGAAACTTCCAAAAACTGACATGAGGAGTCACTGGAGAATCATGATCAAGGAGCTA   79

                                                                   M   K   L        3
CACACTCTGACTTAACTTTATTCTGTGGACAATGAGAGACAACTGCAAGGATTAACAGTGAGAAC ATG AAG CTG     153

 P   L   L   M   F   P   V   C   L   L   W   L   K   D   C   H   C   A   P   T     23
CCA CTT TTG ATG TTT CCC GTG TGT CTG CTA TGG TTG AAA GAC TGT CAT TGT GCA CCT ACT   213

 W   K   D   K   T   A   I   S   E   N   A   N   S   F   S   E   A   G   E   I     43
TGG AAG GAC AAA ACT GCC ATC AGT GAA AAC GCG AAC AGT TTT TCT GAG GCT GGG GAG ATA   273

 D   V   D   G   E   V   K   I   A   L   I   G   I   K   Q   M   K   I   M   M     63
GAC GTA GAT GGA GAG GTG AAG ATA GCT TTG ATT GGC ATT AAA CAG ATG AAA ATC ATG ATG   333

 E   R   R   E   E   E   H   S   K   L   M   K   T   L   K   K   C   K   E   E     83
GAA AGG AGA GAG GAA GAA CAC AGC AAA CTA ATG AAA ACC TTG AAG AAG TGC AAA GAA GAA   393

 K   Q   E   A   L   K   L   M   N   E   V   H   E   H   L   E   E   E   E   S    103
AAG CAG GAG GCC CTG AAA CTT ATG AAT GAA GTT CAT GAA CAC CTG GAG GAG GAA GAA AGC   453

 L   C   Q   V   S   L   A   D   S   W   D   E   C   R   A   C   L   E   S   N    123
TTA TGC CAG GTT TCT CTG GCA GAT TCC TGG GAT GAA TGC AGG GCT TGC CTG GAA AGT AAC   513

 C   M   R   F   D   T   T   C   Q   P   A   W   S   S   V   K   N   M   V   E    143
TGC ATG AGG TTT GAT ACC ACC TGC CAA CCT GCA TGG TCC TCT GTG AAA AAT ATG GTG GAA   573

 Q   F   F   R   K   I   Y   Q   F   L   F   P   L   Q   E   N   D   R   S   G    163
CAG TTT TTC AGG AAG ATC TAT CAG TTT CTG TTT CCT CTC CAG GAA AAT GAC AGA AGT GGC   633

 P   V   S   K   G   V   T   E   E   D   A   Q   V   S   H   I   E   H   V   F    183
CCT GTC AGC AAA GGG GTC ACT GAG GAA GAT GCG CAG GTG TCA CAC ATA GAG CAT GTG TTC   693

 S   Q   L   S   A   D   V   T   S   L   F   N   R   S   L   Y   V   F   K   Q    203
AGC CAG CTG AGC GCA GAT GTG ACA TCT CTC TTC AAC AGA AGC CTT TAC GTC TTC AAA CAG   753

 L   R   R   E   F   D   Q   A   F   Q   S   Y   F   T   S   G   T   D   V   T    223
CTG CGG CGA GAA TTT GAC CAG GCT TTT CAG TCA TAT TTC ACA TCG GGG ACT GAC GTT ACA   813

 E   P   F   F   F   P   S   L   S   K   E   P   A   Y   R   A   D   A   E   P    243
GAG CCT TTC TTT TTT CCA TCT TTG TCC AAG GAG CCA GCC TAC AGA GCA GAT GCT GAG CCA   873

 S   W   A   I   P   N   V   F   Q   L   L   C   N   L   S   F   S   V   Y   Q    263
AGC TGG GCC ATT CCC AAT GTC TTC CAG CTG CTC TGC AAC TTG AGT TTC TCA GTT TAT CAA   933

 S   V   S   E   K   L   I   T   T   L   R   A   T   E   D   P   P   K   Q   D    283
AGT GTC AGT GAA AAA CTC ATC ACA ACC CTG CGT GCC ACA GAG GAC CCT CCA AAA CAA GAC   993

 K   D   S   N   Q   G   G   P   I   S   K   I   L   P   E   Q   D   R   G   S    303
AAA GAC TCC AAC CAG GGA GGC CCG ATT TCA AAG ATA CTA CCT GAG CAA GAC AGA GGC TCA  1053

 D   G   K   L   G   Q   N   L   S   D   C   V   N   F   R   K   R   C   Q   K    323
GAT GGG AAA CTT GGC CAG AAT TTG TCT GAT TGC GTT AAT TTT CGC AAG AGA TGC CAG AAA  1113

 C   Q   D   Y   L   S   D   D   C   P   N   V   P   E   L   Y   R   E   L   N    343
TGC CAG GAT TAT CTA TCT GAT GAC TGC CCT AAT GTG CCT GAA CTA TAC AGA GAA CTC AAT  1173

 E   A   L   R   L   V   S   R   S   N   Q   Q   Y   D   Q   V   V   Q   M   T    363
GAG GCC CTC CGA CTG GTC AGT AGA TCC AAT CAG CAA TAC GAC CAG GTG GTG CAG ATG ACC  1233

 Q   Y   H   L   E   D   T   T   L   L   M   E   K   M   R   E   Q   F   G   W    383
CAG TAT CAC CTG GAA GAC ACC ACG CTT CTG ATG GAG AAG ATG AGA GAG CAG TTT GGC TGG  1293

 V   S   E   L   A   Y   Q   S   P   G   A   E   D   I   F   N   P   V   K   V    403
GTT TCT GAA CTG GCA TAC CAG TCC CCA GGA GCT GAG GAC ATC TTT AAT CCA GTG AAA GTA  1353
```

FIG. 7A

```
 M   V   A   L   S   A   H   E   G   N   S   S   D   Q   D   D   T   V   V   P    423
ATG GTA GCC CTA AGT GCT CAT GAA GGA AAT TCT TCT GAT CAA GAT GAC ACA GTG GTT CCT  1413

 S   S   L   L   P   S   S   N   F   T   L   S   S   P   L   E   K   S   A   G    443
TCA AGC CTC CTG CCT TCC TCT AAC TTC ACA CTC AGC AGC CCT CTT GAA AAG AGT GCT GGC  1473

 N   A   N   F   I   D   H   V   V   E   K   V   L   Q   H   F   K   E   H   F    463
AAC GCT AAC TTC ATT GAT CAC GTG GTA GAG AAG GTT CTT CAG CAC TTT AAG GAG CAC TTT  1533

 K   T   W   *                                                                    467
AAA ACT TGG TAA                                                                  1545

GAAGATTTAGTCCATCCTATAATCAGCAAGAATTACACCTTCGGCCAAGACCTGAGAATTCTGAAAATACAAAGCAGGC  1624

TAACACAATGAACACAGCTGCATGAAAGTTAGGTATATATTAGGAAGCACTATTGGTTTACTTTGTTGAATGGAAGTTT  1703

AATAGCTATTCAAATTGAGTTAATATAAAAATTTCTTCCTAAAAAGTAAAATGTACATATGTAGAATATGATGCATTAG  1782

TTCTTTGTATACTAAATAAATACTGAGTCCCCT                                                1815
```

FIG. 7B

CTTGGAGTCAACTGAGTGTGGACTGAAACTTCCAAAAACTGACATGAGGAGTCACTGGAGAATCATGATCAAGGAGCTA 79

M K L 3
CACACTCTGACTTAACTTTATTCTGTGGACAATGAGAGACAACTGCAAGGATTAACAGTGAGAAC ATG AAG CTG 153

P L L M F P V C L L W L K D C H C A P T 23
CCA CTT TTG ATG TTT CCC GTG TGT CTG CTA TGG TTG AAA GAC TGT CAT TGT GCA CCT ACT 213

W K D K T A I S E N A N S F S E A G E I 43
TGG AAG GAC AAA ACT GCC ATC AGT GAA AAC GCG AAC AGT TTT TCT GAG GCT GGG GAG ATA 273

D V D G E V K I A L I G I K Q M K I M M 63
GAC GTA GAT GGA GAG GTG AAG ATA GCT TTG ATT GGC ATT AAA CAG ATG AAA ATC ATG ATG 333

E R R E E E H S K L M K T L K K C K E E 83
GAA AGG AGA GAG GAA GAA CAC AGC AAA CTA ATG AAA ACC TTG AAG AAG TGC AAA GAA GAA 393

K Q E A L K L M N E V H E H L E E E E S 103
AAG CAG GAG GCC CTG AAA CTT ATG AAT GAA GTT CAT GAA CAC CTG GAG GAG GAA GAA AGC 453

L C Q V S L A D S W D E C R A C L E S N 123
TTA TGC CAG GTT TCT CTG GCA GAT TCC TGG GAT GAA TGC AGG GCT TGC CTG GAA AGT AAC 513

C M R F D T T C Q P A W S S V K N M E N 143
TGC ATG AGG TTT GAT ACC ACC TGC CAA CCT GCA TGG TCC TCT GTG AAA AAT ATG GAA AAT 573

D R S G P V S K G V T E E D A Q V S H I 163
GAC AGA AGT GGC CCT GTC AGC AAA GGG GTC ACT GAG GAA GAT GCG CAG GTG TCA CAC ATA 633

E H V F S Q L S A D V T S L F N R S L Y 183
GAG CAT GTG TTC AGC CAG CTG AGC GCA GAT GTG ACA TCT CTC TTC AAC AGA AGC CTT TAC 693

V F K Q L R R E F D Q A F Q S Y F T S G 203
GTC TTC AAA CAG CTG CGG CGA GAA TTT GAC CAG GCT TTT CAG TCA TAT TTC ACA TCG GGG 753

T D V T E P F F F P S L S K E P A Y R A 223
ACT GAC GTT ACA GAG CCT TTC TTT TTT CCA TCT TTG TCC AAG GAG CCA GCC TAC AGA GCA 813

D A E P S W A I P N V F Q L L C N L S F 243
GAT GCT GAG CCA AGC TGG GCC ATT CCC AAT GTC TTC CAG CTG CTC TGC AAC TTG AGT TTC 873

S V Y Q S V S E K L I T T L R A T E D P 263
TCA GTT TAT CAA AGT GTC AGT GAA AAA CTC ATC ACA ACC CTG CGT GCC ACA GAG GAC CCT 933

P K Q D K D S N Q G G P I S K I L P E Q 283
CCA AAA CAA GAC AAA GAC TCC AAC CAG GGA GGC CCG ATT TCA AAG ATA CTA CCT GAG CAA 993

D R G S D G K L G Q N L S D C V N F R K 303
GAC AGA GGC TCA GAT GGG AAA CTT GGC CAG AAT TTG TCT GAT TGC GTT AAT TTT CGC AAG 1053

R C Q K C Q D Y L S D D C P N V P E L Y 323
AGA TGC CAG AAA TGC CAG GAT TAT CTA TCT GAT GAC TGC CCT AAT GTG CCT GAA CTA TAC 1113

R E L N E A L R L V S R S N Q Q Y D Q V 343
AGA GAA CTC AAT GAG GCC CTC CGA CTG GTC AGT AGA TCC AAT CAG CAA TAC GAC CAG GTG 1173

V Q M T Q Y H L E D T T L L M E K M R E 363
GTG CAG ATG ACC CAG TAT CAC CTG GAA GAC ACC ACG CTT CTG ATG GAG AAG ATG AGA GAG 1233

Q F G W V S E L A Y Q S P G A E D I F N 383
CAG TTT GGC TGG GTT TCT GAA CTG GCA TAC CAG TCC CCA GGA GCT GAG GAC ATC TTT AAT 1293

P V K V M V A L S A H E G N S S D Q D D 403
CCA GTG AAA GTA ATG GTA GCC CTA AGT GCT CAT GAA GGA AAT TCT TCT GAT CAA GAT GAC 1353

FIG. 8A

```
 T   V   V   P   S   S   L   L   P   S   S   N   F   T   L   S   S   P   L   E    423
ACA GTG GTT CCT TCA AGC CTC CTG CCT TCC TCT AAC TTC ACA CTC AGC AGC CCT CTT GAA  1413

 K   S   A   G   N   A   N   F   I   D   H   V   V   E   K   V   L   Q   H   F    443
AAG AGT GCT GGC AAC GCT AAC TTC ATT GAT CAC GTG GTA GAG AAG GTT CTT CAG CAC TTT  1473

 K   E   H   F   K   T   W   *                                                    451
AAG GAG CAC TTT AAA ACT TGG TAA                                                  1497

GAAGATTTAGTCCATCCTATAATCAGCAAGAATTACACCTTCGGCCAAGACCTGAGAATTCTGAAAATACAAAGCAGGC  1576

TAACACAATGAACACAGCTGCATGAAAGTTAGGTATATATTAGGAAGCACTATTGGTTTACTTTGTTGAATGGAAGTTT  1655

AATAGCTATTCAAATTGAGTTAATATAAAAATTTCTTCCTAAAAAGTAAAATGTACATATGTAGAATATGATGCATTAG  1734

TTCTTTGTATACTAAATAAATACTGAGTCCCCT                                                1767
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FIG. 8B

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CTTGGAGTCAACTGAGTGTGGACTGAAACTTCCAAAAACTGACATGAGGAGTCACTGGAGAATCATGATCAAGGAGCTA   79

                                                                     M   K   L      3
CACACTCTGACTTAACTTTATTCTGTGGACAATGAGAGACAACTGCAAGGATTAACAGTGAGAAC ATG AAG CTG     153

 P   L   L   M   F   P   V   C   L   L   W   L   K   D   C   H   C   A   P   T     23
CCA CTT TTG ATG TTT CCC GTG TGT CTG CTA TGG TTG AAA GAC TGT CAT TGT GCA CCT ACT   213

 W   K   D   K   T   A   I   S   E   N   A   N   S   F   S   E   A   G   E   I     43
TGG AAG GAC AAA ACT GCC ATC AGT GAA AAC GCG AAC AGT TTT TCT GAG GCT GGG GAG ATA   273

 D   V   D   G   E   V   K   I   A   L   I   G   I   K   Q   M   K   I   M   M     63
GAC GTA GAT GGA GAG GTG AAG ATA GCT TTG ATT GGC ATT AAA CAG ATG AAA ATC ATG ATG   333

 E   R   R   E   E   E   H   S   K   L   M   K   T   L   K   K   C   K   E   E     83
GAA AGG AGA GAG GAA GAA CAC AGC AAA CTA ATG AAA ACC TTG AAG AAG TGC AAA GAA GAA   393

 K   Q   E   A   L   K   L   M   N   E   V   H   E   H   L   E   E   E   E   S    103
AAG CAG GAG GCC CTG AAA CTT ATG AAT GAA GTT CAT GAA CAC CTG GAG GAG GAA GAA AGC   453

 L   C   Q   V   S   L   A   D   S   W   D   E   C   R   A   C   L   E   S   N    123
TTA TGC CAG GTT TCT CTG GCA GAT TCC TGG GAT GAA TGC AGG GCT TGC CTG GAA AGT AAC   513

 C   M   R   F   D   T   T   C   Q   P   A   W   S   S   V   K   N   M   E   P    143
TGC ATG AGG TTT GAT ACC ACC TGC CAA CCT GCA TGG TCC TCT GTG AAA AAT ATG GAG CCA   573

 A   Y   R   A   D   A   E   P   S   W   A   I   P   N   V   F   Q   L   L   C    163
GCC TAC AGA GCA GAT GCT GAG CCA AGC TGG GCC ATT CCC AAT GTC TTC CAG CTG CTC TGC   633

 N   L   S   F   S   V   Y   Q   S   V   S   E   K   L   I   T   T   L   R   A    183
AAC TTG AGT TTC TCA GTT TAT CAA AGT GTC AGT GAA AAA CTC ATC ACA ACC CTG CGT GCC   693

 T   E   D   P   P   K   Q   D   K   D   S   N   Q   G   G   P   I   S   K   I    203
ACA GAG GAC CCT CCA AAA CAA GAC AAA GAC TCC AAC CAG GGA GGC CCG ATT TCA AAG ATA   753

 L   P   E   Q   D   R   G   S   D   G   K   L   G   Q   N   L   S   D   C   V    223
CTA CCT GAG CAA GAC AGA GGC TCA GAT GGG AAA CTT GGC CAG AAT TTG TCT GAT TGC GTT   813

 N   F   R   K   R   C   Q   K   C   Q   D   Y   L   S   D   D   C   P   N   V    243
AAT TTT CGC AAG AGA TGC CAG AAA TGC CAG GAT TAT CTA TCT GAT GAC TGC CCT AAT GTG   873

 P   E   L   Y   R   E   L   N   E   A   L   R   L   V   S   R   S   N   Q   Q    263
CCT GAA CTA TAC AGA GAA CTC AAT GAG GCC CTC CGA CTG GTC AGT AGA TCC AAT CAG CAA   933

 Y   D   Q   V   V   Q   M   T   Q   Y   H   L   E   D   T   T   L   L   M   E    283
TAC GAC CAG GTG GTG CAG ATG ACC CAG TAT CAC CTG GAA GAC ACC ACG CTT CTG ATG GAG   993

 K   M   R   E   Q   F   G   W   V   S   E   L   A   Y   Q   S   P   G   A   E    303
AAG ATG AGA GAG CAG TTT GGC TGG GTT TCT GAA CTG GCA TAC CAG TCC CCA GGA GCT GAG  1053

 D   I   F   N   P   V   K   V   M   V   A   L   S   A   H   E   G   N   S   S    323
GAC ATC TTT AAT CCA GTG AAA GTA ATG GTA GCC CTA AGT GCT CAT GAA GGA AAT TCT TCT  1113

 D   Q   D   D   T   V   V   P   S   S   L   L   P   S   S   N   F   T   L   S    343
GAT CAA GAT GAC ACA GTG GTT CCT TCA AGC CTC CTG CCT TCC TCT AAC TTC ACA CTC AGC  1173

 S   P   L   E   K   S   A   G   N   A   N   F   I   D   H   V   V   E   K   V    363
AGC CCT CTT GAA AAG AGT GCT GGC AAC GCT AAC TTC ATT GAT CAC GTG GTA GAG AAG GTT  1233

 L   Q   H   F   K   E   H   F   K   T   W   *                                    375
CTT CAG CAC TTT AAG GAG CAC TTT AAA ACT TGG TAA                                  1269

GAAGATTTAGTCCATCCTATAATCAGCAAGAATTACACCTTCGGCCAAGACCTGAGAATTCTGAAAATACAAAGCAGGC  1348
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FIG. 9A

TAACACAATGAACACAGCTGCATGAAAGTTAGGTATATATTAGGAAGCACTATTGGTTTACTTTGTTGAATGGAAGTTT 1427

AATAGCTATTCAAATTGAGTTAATATAAAAATTTCTTCCTAAAAAGTAAAATGTACATATGTAGAATATGATGCATTAG 1506

TTCTTTGTATACTAAATAAATACTGAGTCCCCT 1539

FIG. 9B

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CTTGGAGTCAACTGAGTGTGGACTGAAACTTCCAAAAACTGACATGAGGAGTCACTGGAGAATCATGATCAAGGAGCTA   79

                                                                  M   K   L      3
CACACTCTGACTTAACTTTATTCTGTGGACAATGAGAGACAACTGCAAGGATTAACAGTGAGAAC ATG AAG CTG   153

 P   L   L   M   F   P   V   C   L   L   W   L   K   D   C   H   C   A   P   T    23
CCA CTT TTG ATG TTT CCC GTG TGT CTG CTA TGG TTG AAA GAC TGT CAT TGT GCA CCT ACT  213

 W   K   D   K   T   A   I   S   E   N   A   N   S   F   S   E   A   G   E   I    43
TGG AAG GAC AAA ACT GCC ATC AGT GAA AAC GCG AAC AGT TTT TCT GAG GCT GGG GAG ATA  273

 D   V   D   G   E   V   K   I   A   L   I   G   I   K   Q   M   K   I   M   M    63
GAC GTA GAT GGA GAG GTG AAG ATA GCT TTG ATT GGC ATT AAA CAG ATG AAA ATC ATG ATG  333

 E   R   R   E   E   E   H   S   K   L   M   K   T   L   K   K   C   K   E   E    83
GAA AGG AGA GAG GAA GAA CAC AGC AAA CTA ATG AAA ACC TTG AAG AAG TGC AAA GAA GAA  393

 K   Q   E   A   L   K   L   M   N   E   V   H   E   H   L   E   E   E   E   S   103
AAG CAG GAG GCC CTG AAA CTT ATG AAT GAA GTT CAT GAA CAC CTG GAG GAG GAA GAA AGC  453

 L   C   Q   V   S   L   A   D   S   W   D   E   C   R   A   C   L   E   S   N   123
TTA TGC CAG GTT TCT CTG GCA GAT TCC TGG GAT GAA TGC AGG GCT TGC CTG GAA AGT AAC  513

 C   M   R   F   D   T   T   C   Q   P   A   W   S   S   V   K   N   M   P   A   143
TGC ATG AGG TTT GAT ACC ACC TGC CAA CCT GCA TGG TCC TCT GTG AAA AAT ATG CCA GCC  573

 Y   R   A   D   A   E   P   S   W   A   I   P   N   V   F   Q   L   L   C   N   163
TAC AGA GCA GAT GCT GAG CCA AGC TGG GCC ATT CCC AAT GTC TTC CAG CTG CTC TGC AAC  633

 L   S   F   S   V   Y   Q   S   V   S   E   K   L   I   T   T   L   R   A   T   183
TTG AGT TTC TCA GTT TAT CAA AGT GTC AGT GAA AAA CTC ATC ACA ACC CTG CGT GCC ACA  693

 E   D   P   P   K   Q   D   K   D   S   N   Q   G   G   P   I   S   K   I   L   203
GAG GAC CCT CCA AAA CAA GAC AAA GAC TCC AAC CAG GGA GGC CCG ATT TCA AAG ATA CTA  753

 P   E   Q   D   R   G   S   D   G   K   L   G   Q   N   L   S   D   C   V   N   223
CCT GAG CAA GAC AGA GGC TCA GAT GGG AAA CTT GGC CAG AAT TTG TCT GAT TGC GTT AAT  813

 F   R   K   R   C   Q   K   C   Q   D   Y   L   S   D   D   C   P   N   V   P   243
TTT CGC AAG AGA TGC CAG AAA TGC CAG GAT TAT CTA TCT GAT GAC TGC CCT AAT GTG CCT  873

 E   L   Y   R   E   L   N   E   A   L   R   L   V   S   R   S   N   Q   Q   Y   263
GAA CTA TAC AGA GAA CTC AAT GAG GCC CTC CGA CTG GTC AGT AGA TCC AAT CAG CAA TAC  933

 D   Q   V   V   Q   M   T   Q   Y   H   L   E   D   T   T   L   L   M   E   K   283
GAC CAG GTG GTG CAG ATG ACC CAG TAT CAC CTG GAA GAC ACC ACG CTT CTG ATG GAG AAG  993

 M   R   E   Q   F   G   W   V   S   E   L   A   Y   Q   S   P   G   A   E   D   303
ATG AGA GAG CAG TTT GGC TGG GTT TCT GAA CTG GCA TAC CAG TCC CCA GGA GCT GAG GAC 1053

 I   F   N   P   V   K   V   M   V   A   L   S   A   H   E   G   N   S   S   D   323
ATC TTT AAT CCA GTG AAA GTA ATG GTA GCC CTA AGT GCT CAT GAA GGA AAT TCT TCT GAT 1113

 Q   D   D   T   V   V   P   S   S   L   L   P   S   S   N   F   T   L   S   S   343
CAA GAT GAC ACA GTG GTT CCT TCA AGC CTC CTG CCT TCC TCT AAC TTC ACA CTC AGC AGC 1173

 P   L   E   K   S   A   G   N   A   N   F   I   D   H   V   V   E   K   V   L   363
CCT CTT GAA AAG AGT GCT GGC AAC GCT AAC TTC ATT GAT CAC GTG GTA GAG AAG GTT CTT 1233

 Q   H   F   K   E   H   F   K   T   W   *                                       374
CAG CAC TTT AAG GAG CAC TTT AAA ACT TGG TAA                                     1266

GAAGATTTAGTCCATCCTATAATCAGCAAGAATTACACCTTCGGCCAAGACCTGAGAATTCTGAAAATACAAAGCAGGC 1345
```

FIG. 10A

TAACACAATGAACACAGCTGCATGAAAGTTAGGTATATATTAGGAAGCACTATTGGTTTACTTTGTTGAATGGAAGTTT 1424

AATAGCTATTCAAATTGAGTTAATATAAAAATTTCTTCCTAAAAAGTAAAATGTACATATGTAGAATATGATGCATTAG 1503

TTCTTTGTATACTAAATAAATACTGAGTCCCCT 1536

FIG. 10B

M K P P L L V F 8
GCAACCTCGTTGGTGAGAGCCTGCAGTTAGTGTCACGGCGGAAAC ATG AAG CCG CCA CTC TTG GTG TTT 69

I V Y L L R L R D C Q C A P T G K D R T 28
ATT GTG TAT CTG CTG CGG CTG AGA GAC TGT CAG TGT GCG CCT ACA GGG AAG GAC CGA ACT 129

S I R E D P K G F S K A G E I D V D E E 48
TCC ATC CGT GAA GAC CCG AAG GGT TTT TCC AAG GCT GGG GAG ATA GAC GTA GAT GAA GAG 189

V K K A L I G M K Q M K I L M E R R E E 68
GTG AAG AAG GCT TTG ATT GGC ATG AAG CAG ATG AAA ATC CTG ATG GAA AGA AGA GAG GAG 249

E H S K L M R T L K K C R E E K Q E A L 88
GAA CAT AGC AAA CTA ATG AGA ACA CTG AAG AAA TGC AGA GAA GAA AAG CAG GAG GCC CTG 309

K L M N E V Q E H L E E E E R L C Q V S 108
AAG CTT ATG AAT GAA GTT CAA GAA CAT CTA GAA GAG GAA GAA AGG CTA TGC CAG GTG TCT 369

L M G S W D E C K S C L E S D C M R F Y 128
CTG ATG GGT TCC TGG GAC GAA TGC AAA TCT TGC CTG GAA AGT GAC TGC ATG AGA TTT TAT 429

T T C Q S S W S S M K S T I E R V F R K 148
ACA ACC TGC CAA AGC AGT TGG TCC TCT ATG AAA TCC ACG ATT GAA CGG GTT TTC CGG AAG 489

I Y Q F L F P F H E D D E K E L P V G E 168
ATA TAT CAG TTT CTC TTT CCT TTC CAT GAA GAC GAT GAA AAA GAG CTT CCT GTT GGT GAG 549

K F T E E D V Q L M Q I E N V F S Q L T 188
AAG TTC ACT GAG GAA GAT GTA CAG CTG ATG CAG ATA GAG AAT GTG TTC AGC CAG CTG ACC 609

V D V G F L Y N M S F H V F K Q M Q Q E 208
GTG GAT GTG GGA TTT CTC TAT AAC ATG AGC TTT CAC GTC TTC AAA CAG ATG CAG CAA GAA 669

F D L A F Q S Y F M S D T D S M E P Y F 228
TTT GAC CTG GCT TTT CAA TCA TAC TTT ATG TCA GAC ACA GAC TCC ATG GAG CCT TAC TTT 729

F P A F S K E P A K K A H P M Q S W D I 248
TTT CCA GCT TTT TCC AAA GAG CCA GCA AAA AAA GCA CAT CCT ATG CAG AGT TGG GAC ATT 789

P S F F Q L F C N F S L S V Y Q S V S A 268
CCC AGC TTC TTC CAG CTG TTT TGT AAT TTC AGC CTC TCT GTT TAT CAA AGT GTC AGC GCA 849

T V T E M L K A I E D L S K Q D K D S A 288
ACA GTT ACA GAG ATG CTG AAG GCC ATT GAG GAC TTA TCC AAA CAA GAC AAA GAT TCT GCC 909

H G G P S S T T W P V R G R G L C G E P 308
CAC GGT GGA CCG AGT TCC ACG ACG TGG CCT GTG CGG GGC AGA GGG CTG TGT GGA GAA CCT 969

G Q N S S E C L Q F H A R C Q K C Q D Y 328
GGC CAG AAC TCG TCC GAA TGT CTC CAA TTT CAT GCA AGA TGC CAG AAA TGT CAG GAT TAC 1029

L W A D C P A V P E L Y T K A D E A L E 348
CTA TGG GCA GAC TGC CCT GCT GTT CCT GAA CTA TAC ACA AAG GCG GAT GAG GCC CTT GAG 1089

L V N I S N Q Q Y A Q V L Q M T Q H H L 368
TTG GTC AAC ATA TCC AAT CAG CAG TAT GCC CAG GTA CTC CAG ATG ACC CAG CAT CAC TTG 1149

E D T T Y L M E K M R E Q F G W V T E L 388
GAG GAC ACC ACG TAT CTG ATG GAG AAG ATG AGA GAG CAG TTT GGT TGG GTA ACA GAG CTG 1209

A S Q T P G S E N I F S F I K V V P G V 408
GCC AGC CAG ACC CCA GGA AGC GAG AAC ATC TTC AGT TTC ATA AAG GTA GTT CCA GGT GTT 1269

H E G N F S K Q D E K M I D I S I L P S 428

FIG. 11A

```
CAC GAA GGA AAT TTC TCC AAA CAA GAT GAA AAG ATG ATA GAC ATA AGC ATT CTG CCT TCC  1329

 S   N   F   T   L   T   I   P   L   E   E   S   A   E   S   S   D   F   I   S   448
TCT AAT TTC ACA CTC ACC ATC CCT CTT GAA GAA AGT GCT GAG AGT TCC GAC TTC ATT AGC  1389

 Y   M   L   A   K   A   V   Q   H   F   K   E   H   F   K   S   W   *           466
TAC ATG CTG GCC AAA GCT GTA CAG CAT TTT AAG GAA CAT TTT AAA TCT TGG TAA          1443

GCAGAGTATTTGATTAGGGACGTTTGCTGATAGGAATAGATGGTTCTTAAAAGGGAAAAATGACAAAACTAGCTTTTGA  1522

ATACCTTGAAAACGTATTCAACCTCATTAATAATCAAAGGCATGAAAACTAAGACAAGTTAGCAGTTTTTACCTATTGA  1601

ATTTTCAAATTAAAAAAAAAAATCCTGATAGAATGCAATGAAATGAGAATTCTTATATGTGATTGCCAGAAACAAACTG  1680

GTTTTGTCTTTTTGAAAAGTTATTCAATTATACATATCAAGAGTCATCAAATTTCTTTTTAATATAATAATTCCACTTC  1759

TGGAATCAATCCAAAGGAGTAAATCTAAAATTGAATTGAAGTTCCCACCCCAAGATCAATATTTGCAAATTATTTAAAA  1838

TAGTAAACTGTTAAAAACTGAATGTCATCTGAATGTCTAAAAACCAGAAATGGTTAAAAGCTGTGGCTAAATATGCTCC  1917

AAATATCTTATAAAACCATTAAAAATATTTATAAAATTTAAATCATGACATGACATCTGCTGGAACAAGAGTTTATTCT  1996

AAGCCTATCTATAAGGCAAATATTATTATTACTATCTTCCAGAAAAGAAACTTGAGACTCAGGGTCCAAGTGTTAGTTG  2075

CTCAGTCATGTCTGACTCTTTGGGACCCCTTGGACTGTAGCCCACCAGGCTCCTCTGTCCGTGGGATTCTTCAGACAGG  2154

AATACTGGGGCAGGTTGCTATTTCCTTCTCCAGGAAATCTTCCCTATCCAGGGATGGAACCCAGGTCTCCTGCATTGCA  2233

GGTAGATGCTTTACTATCTGAGCAACCAAATGAATTACTCAAGTCAGTAGGGGGTAGAGGCAAATTTTAACTTAGTTTT  2312

CTCTGAATCATAATTGCCACATTAAACTGGTTCCTGTTGGGACATTTGGTTGAAAAAAATAAAGTGAAAAATGAGTATA  2391

AAACTCTATAAATGTAATGATCAAAACGAAAAAAAATCTACAATCTGCATTAAAAATAAAAAGGGTTGGCAGG        2464
```

FIG. 11B

M K P P L 5
CAGAAGCTGGTGGCAACCTCGTTGGTGAGAGCCTGCAGTTAGTGTCACGGCGGAAAC ATG AAG CCG CCA CTC 72

L V F I V Y L L R L R D C Q C A P T G K 25
TTG GTG TTT ATT GTG TAT CTG CTG CGG CTG AGA GAC TGT CAG TGT GCG CCT ACA GGG AAG 132

D R T S I R E D P K G F S K A G E I D V 45
GAC CGA ACT TCC ATC CGT GAA GAC CCG AAG GGT TTT TCC AAG GCT GGG GAG ATA GAC GTA 192

D E E V K K A L I G M K Q M K I L M E R 65
GAT GAA GAG GTG AAG AAG GCT TTG ATT GGC ATG AAG CAG ATG AAA ATC CTG ATG GAA AGA 252

R E E E H S K L M R T L K K C R E E K Q 85
AGA GAG GAG GAA CAT AGC AAA CTA ATG AGA ACA CTG AAG AAA TGC AGA GAA GAA AAG CAG 312

E A L K L M N E V Q E H L E E E E R L C 105
GAG GCC CTG AAG CTT ATG AAT GAA GTT CAA GAA CAT CTA GAA GAG GAA GAA AGG CTA TGC 372

Q V S L M G S W D E C K S C L E S D C M 125
CAG GTG TCT CTG ATG GGT TCC TGG GAC GAA TGC AAA TCT TGC CTG GAA AGT GAC TGC ATG 432

R F Y T T C Q S S W S S M K S T I E R V 145
AGA TTT TAT ACA ACC TGC CAA AGC AGT TGG TCC TCT ATG AAA TCC ACG ATT GAA CGG GTT 492

F R K I Y Q F L F P F H E D D E K E L P 165
TTC CGG AAG ATA TAT CAG TTT CTC TTT CCT TTC CAT GAA GAC GAT GAA AAA GAG CTT CCT 552

V G E K F T E E D V Q L M Q I E N V F S 185
GTT GGT GAG AAG TTC ACT GAG GAA GAT GTA CAG CTG ATG CAG ATA GAG AAT GTG TTC AGC 612

Q L T V D V G F L Y N M S F H V F K Q M 205
CAG CTG ACC GTG GAT GTG GGA TTT CTC TAT AAC ATG AGC TTT CAC GTC TTC AAA CAG ATG 672

Q Q E F D L A F Q S Y F M S D T D S M E 225
CAG CAA GAA TTT GAC CTG GCT TTT CAA TCA TAC TTT ATG TCA GAC ACA GAC TCC ATG GAG 732

P Y F F P A F S K E P A K K A H P M Q S 245
CCT TAC TTT TTT CCA GCT TTT TCC AAA GAG CCA GCA AAA AAA GCA CAT CCT ATG CAG AGT 792

W D I P S F F Q L F C N F S L S V Y Q S 265
TGG GAC ATT CCC AGC TTC TTC CAG CTG TTT TGT AAT TTC AGC CTC TCT GTT TAT CAA AGT 852

V S A T V T E M L K A I E D L S K Q D K 285
GTC AGC GCA ACA GTT ACA GAG ATG CTG AAG GCC ATT GAG GAC TTA TCC AAA CAA GAC AAA 912

D S A H G G P S S T T W P V R G R G L C 305
GAT TCT GCC CAC GGT GGA CCG AGT TCC ACG ACG TGG CCT GTG CGG GGC AGA GGG CTG TGT 972

G E P G Q N S S E C L Q F H A R C Q K C 325
GGA GAA CCT GGC CAG AAC TCG TCC GAA TGT CTC CAA TTT CAT GCA AGA TGC CAG AAA TGT 1032

Q D Y L W A D C P A V P E L Y T K A D E 345
CAG GAT TAC CTA TGG GCA GAC TGC CCT GCT GTT CCT GAA CTA TAC ACA AAG GCG GAT GAG 1092

A L E L V N I S N Q Q Y A Q V L Q M T Q 365
GCC CTT GAG TTG GTC AAC ATA TCC AAT CAG CAG TAT GCC CAG GTA CTC CAG ATG ACC CAG 1152

H H L E D T T Y L M E K M R E Q F G W V 385
CAT CAC TTG GAG GAC ACC ACG TAT CTG ATG GAG AAG ATG AGA GAG CAG TTT GGT TGG GTA 1212

T E L A S Q T P G S E N I F S F I K V V 405
ACA GAG CTG GCC AGC CAG ACC CCA GGA AGC GAG AAC ATC TTC AGT TTC ATA AAG GTA GTT 1272

FIG. 12A

```
 P   G   V   H   E   G   N   F   S   K   Q   D   E   K   M   I   D   I   S   I    425
CCA GGT GTT CAC GAA GGA AAT TTC TCC AAA CAA GAT GAA AAG ATG ATA GAC ATA AGC ATT  1332

 L   P   S   S   N   F   T   L   T   I   P   L   E   E   S   A   E   S   S   D    445
CTG CCT TCC TCT AAT TTC ACA CTC ACC ATC CCT CTT GAA GAA AGT GCT GAG AGT TCC GAC  1392

 F   I   S   Y   M   L   A   K   A   V   Q   H   F   K   E   H   F   K   S   W    465
TTC ATT AGC TAC ATG CTG GCC AAA GCT GTA CAG CAT TTT AAG GAA CAT TTT AAA TCT TGG  1452

 *                                                                                466
TAA                                                                              1455

GCAGAGTATTTGATTAGGGACGTTTGCTGATAGGAATAGATGGTTCTTAAAAGGGAAAAATGACAAAACTAGCTTTTGA  1534

ATACCTTGAAAACGTATTCAACCTCATTAATAATCAAAGGCATGAAAACTAAGACAAGTTAGCAGTTTTTACCTATTGA  1613

ATTTTCAAATTAAAAAAAAAAATCCTGATAGAATGCAATGAAATGAGAATTCTTATATGTGATTGCCAGAAACAAACTG  1692

GTTTTGTCTTTTTGAAAAGTTATTCAATTATACATATCAAGAGTCATCAAATTTCTTTTTAATATAATAATTCCACTTC  1771

TGGAATCAATCCAAAGGAGTAAATCTAAAATTGAATTGAAGTTCCCACCCCAAGATCAATATTTGCAAATTATTTAAAA  1850

TAGTAAACTGTTAAAAACTGAATGTCATCTGAATGTCTAAAAACCAGAAATGGTTAAAAGCTGTGGCTAAATATGCTCC  1929

AAATATCTTATAAAACCATTAAAAATATTTATAAAATTTAAATCATGACATGACATCTGCTGGAACAAGAGTTTATTCT  2008

AAGCCTATCTATAAGGCAAATATTATTATTACTATCTTCCAGAAAAGAAACTTGAGACTCAGGGTCCAAGTGTTAGTTG  2087

CTCAGTCATGTCTGACTCTTTGAGACCCCTTGGACTGTGGCCCACCAGGCTCCTCTGTCCATGGGATTCTTCAGACAAG  2166

AATACTGGAGCAGGTTGCTATTTCCTTCTCCAGGAAATCTTCCCTATCCAGGGATGGAACCCAGGTCTCCTGCATTGCA  2245

GGTAGATGCTTTACTATCTGAGCAACCAAATGAATTACTCAAGTCAGTAGGGGGTAGAGGCAAATTTTAACTTAGTTTT  2324

CTCTGAATCATAATTGCCACATTAAACTGGTTCCTGTTGGGACATTTGGTTGAAAAAAATAAAGTGAAAAATGAGTATA  2403

AAACTCTATAAATGTAATGATCAAAACGAAAAAAAATCTACAATCTGCATTAAAAATAAAAAGGGTTGGCAGGAATTAC  2482

GGTTGGAAATGGATGATTTTTTTTAACCTTTTCATCTTTTGATATTTTACAATTTTCTATAATGAATAAATAATTTTGA  2561

GATTTCAAATTAGAAGATATGTTGCTAAAATAGCTAGGTAAATGTAGATTGAACACTGTATCAATGTGTTCTCATCTTT  2640

AAACTTTAGTATAAGTACTTCTATTCCATGGTAATCCTACAGTAAGACGAAATGTAAATCTGTTCGGTCTACAGGAAAA  2719

ACAACTAAATGACATTTCAGACGTACATTACCATCTCTGTTAGGATAATCTTCTGAATTAATGGCACAATTAGAACTGT  2798

ACATAGTATTCTCCTTTGGTAAAATGGTCAATCTTAAAGAAGCATTAAATGTTAATTCTAAGTTATTACTCATAAGGGA  2877

CCTTGTAGGTAGGTCCCTATCAATGTATAATTAAGCTGGGTATTTCTAGATTCGCTGCCTCTCCCTTTATCTCTGAATG  2956

TTGGAGAGGTTGTTGGTCATCAATCAACCAATATCTTTTTAGCATCTTCTAAGTGAAGGC                     3016
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FIG. 12B

M K 2

GTGAAGGTCCTTACAGAAGCTGGTGGCAACCTCGTTGGTGAGAGCCTGCAGTTAGTGTCACGGCGGAAAC ATG AAG 76

P P I L V F I V Y L L Q L R D C Q C A P 22

CCG CCA ATC TTG GTG TTT ATC GTG TAT CTG CTG CAG CTG AGA GAC TGT CAG TGT GCG CCT 136

T G K D R T S I R E D P K G F S K A G E 42

ACA GGG AAG GAC CGA ACT TCC ATC CGT GAA GAC CCG AAG GGT TTT TCC AAG GCT GGG GAG 196

I D V D E E V K K A L I G M K Q M K I L 62

ATA GAC GTA GAT GAA GAG GTG AAG AAG GCT TTG ATT GGC ATG AAG CAG ATG AAA ATC CTG 256

M E R R E E E H S K L M R T L K K C R E 82

ATG GAA AGA AGA GAG GAG GAA CAT AGC AAA CTA ATG AGA ACC CTG AAG AAA TGC AGA GAA 316

E K Q E A L K L M N E V Q E H L E E E E 102

GAA AAG CAG GAG GCC CTG AAG CTT ATG AAT GAA GTT CAA GAA CAT CTA GAA GAG GAA GAA 376

R L C Q V S L M G S W D E C K S C L E S 122

AGG CTA TGC CAG GTG TCT CTG ATG GGT TCC TGG GAC GAA TGC AAA TCT TGC CTG GAA AGT 436

D C M R F Y T T C Q S S W S S M K S T I 142

GAC TGC ATG AGA TTT TAT ACA ACC TGC CAA AGC AGT TGG TCC TCT ATG AAA TCC ACG ATT 496

E R V F R K I Y Q F L F P F H E D D E K 162

GAA CGG GTT TTC CGG AAG ATA TAT CAG TTT CTC TTT CCT TTC CAT GAA GAC GAT GAA AAA 556

E L P V G E K F T E E D V Q L M Q I E N 182

GAG CTT CCT GTT GGT GAG AAG TTC ACT GAG GAA GAT GTA CAG CTG ATG CAG ATA GAG AAT 616

V F S Q L T V D V G F L Y N M S F H V F 202

GTG TTC AGC CAG CTG ACC GTG GAC GTG GGA TTT CTC TAT AAC ATG AGC TTT CAC GTC TTC 676

K Q M Q Q E F D L A F Q S Y F M S D T D 222

AAA CAG ATG CAG CAA GAA TTT GAC CTG GCT TTT CAA TCA TAC TTT ATG TCA GAC ACA GAC 736

S M E P Y F F P A F S K E P A K K A H P 242

TCC ATG GAG CCT TAC TTT TTT CCA GCT TTT TCC AAA GAG CCA GCA AAA AAA GCA CAT CCT 796

M Q S W D I P S F F Q L F C N F S L S V 262

ATG CAG AGT TGG GAC ATT CCC AGC TTC TTC CAG CTG TTT TGT AAT TTC AGC CTC TCT GTT 856

Y Q S V S A T V T E M L K A I E D L S K 282

TAT CAA AGT GTC AGC GCA ACA GTT ACA GAG ATG CTG AAG GCC ATT GAG GAC TTA TCC AAA 916

Q D K D S A H G G P S S T T W P V R G R 302

CAA GAC AAA GAT TCT GCC CAC GGT GGA CCG AGT TCC ACG ACG TGG CCT GTG CGG GGC AGA 976

G L C G E P G Q N S S E C L Q F H A R C 322

GGG CTG TGT GGA GAA CCT GGC CAG AAC TCG TCC GAA TGT CTC CAA TTT CAT GCA AGA TGC 1036

Q K C Q D Y L W A D C P A V P E L Y T K 342

CAG AAA TGT CAG GAT TAC CTA TGG GCA GAC TGC CCT GCT GTT CCT GAA CTA TAC ACA AAG 1096

A D E A L E L V N I S N Q Q Y A Q V L Q 362

GCG GAT GAG GCC CTT GAG TTG GTC AAC ATA TCC AAT CAG CAG TAT GCC CAG GTA CTC CAG 1156

M T Q H H L E D T T Y L M E K M R E Q F 382

ATG ACC CAG CAT CAC TTG GAG GAC ACC ACG TAT CTG ATG GAG AAG ATG AGA GAG CAG TTT 1216

G W V T E L A S Q T P G S E N I F S F I 402

GGT TGG GTA ACA GAG CTG GCC AGC CAG ACC CCA GGA AGC GAG AAC ATC TTC AGT TTC ATA 1276

K V V P G V H E G N F S K Q D E K M I D 422

FIG. 13A

AAG GTA GTT CCA GGT GTT CAC GAA GGA AAT TTC TCC AAA CAA GAT GAA AAG ATG ATA GAC 1336

I S I L P S S N F T L T I P L E E S A E 442
ATA AGC ATT CTG CCT TCC TCT AAT TTC ACA CTC ACC ATC CCT CTT GAA GAA AGT GCT GAG 1396

S S D F I S Y M L A K A V Q H F K E H F 462
AGT TCC GAC TTC ATT AGC TAC ATG CTG GCC AAA GCT GTA CAG CAT TTT AAG GAA CAT TTT 1456

K S W * 466
AAA TCT TGG TAA 1468

GCAGAGTATTTGATTAGGGACGTTTGCTGATAGGAATAGATGGTTCTTAAAAGGGAAAAATGACAAAACTAGCTTTTGA 1547

ATACCTTGAAAACGTATTCAACCTCATTAATAATCAAAGGCATGAAAACTAAGACAAGTTAGCAGTTTTTACCTATTGA 1626

ATTTTCAAATTAAAAAAAAAATCCTGATAGAATGCAATGAAATGAGAATTCTTATATGTGATTGCCAGAAACAAACTGG 1705

TTTTGTCTTTTTGAAAAGTTATTCAATTATACATATCAAGAGTCATCAAATTTCTTTTTAATATAATAATTCCACTTCT 1784

GGAATCAATCCAAAGGAGTAAATCTAAAATTGAATTGAAGTTCCCACCCCAAGATCAATATTTGCAAATTATTTAAAAT 1863

AGTAAACTGTTAAAAACTGAATGTCATCTGAATGTCTAAAAACCAGAAATGGTTAAAAGCTGTGGCTAAATATGCTCCA 1942

AATATCTTATAAAACCATTAAAAATATTTATAAAATTTAAATCATGACATGACATCTGCTGGAACAAGAGTTTATTCTA 2021

AGCCTATCTATAAGGCAAATATTATTATTACTATCTTCCAGAAAAGAAACTTGAGACTCAGGGTCCAAGTGTTAGTTGC 2100

TCAGTCATGTCTGACTCTTTGAGACCCCTTGGACTGTAGCCCACCAGGCTCCTCTGTCCATGGGATTCTTCAGACAAGA 2179

ATACTGGAGCAGGTTGCTATTTCCTTCTCCAGGAAATCTTCCCTATCCAGGGATGGAACCCAGGTCTCCTGCATTGCAG 2258

GTAGATGCTTTACTATCTGAGCAACCAAATGAATTACTCAAGTCAGTAGGGGGTAGAGGCAAATTTTAACTTAGTTTTC 2337

TCTGAATCATAATTGCCACATTAAACTGGTTCCTGTTGGGACATTTGGTTGAAAAAAATAAAGTGAAAAATGAGTATAA 2416

AACTCTATAAATGTAATGATCAAAACGAAAAAAAATCTACAATCTGCATTAAAAATAAAAAGGGTTGGCAGG 2488

FIG. 13B

	C T T G G A G T C A A C T G A G T G T G G A C T G A A A C T T C C A A A A A C T	Majority
	10 20 30 40	
1	C T T G G A G T C A A C T G A G T G T G G A C T G A A A C T T C C A A A A A C T	gphkng1815-1.
1	C T T G G A G T C A A C T G A G T G T G G A C T G A A A C T T C C A A A A A C T	gp7b-1.
1	C T T G G A G T C A A C T G A G T G T G G A C T G A A A C T T C C A A A A A C T	gp7c-1.
1	C T T G G A G T C A A C T G A G T G T G G A C T G A A A C T T C C A A A A A C T	gp7d-1.
	G A C A T G A G G A G T C A C T G G A G A A T C A T G A T C A A G G A G C T A C	Majority
	50 60 70 80	
41	G A C A T G A G G A G T C A C T G G A G A A T C A T G A T C A A G G A G C T A C	gphkng1815-1.
41	G A C A T G A G G A G T C A C T G G A G A A T C A T G A T C A A G G A G C T A C	gp7b-1.
41	G A C A T G A G G A G T C A C T G G A G A A T C A T G A T C A A G G A G C T A C	gp7c-1.
41	G A C A T G A G G A G T C A C T G G A G A A T C A T G A T C A A G G A G C T A C	gp7d-1.
	A C A C T C T G A C T T A A C T T T A T T C T G T G G A C A A T G A G A G A C A	Majority
	90 100 110 120	
81	A C A C T C T G A C T T A A C T T T A T T C T G T G G A C A A T G A G A G A C A	gphkng1815-1.
81	A C A C T C T G A C T T A A C T T T A T T C T G T G G A C A A T G A G A G A C A	gp7b-1.
81	A C A C T C T G A C T T A A C T T T A T T C T G T G G A C A A T G A G A G A C A	gp7c-1.
81	A C A C T C T G A C T T A A C T T T A T T C T G T G G A C A A T G A G A G A C A	gp7d-1.
	A C T G C A A G G A T T A A C A G T G A G A A C A T G A A G C T G C C A C T T T	Majority
	130 140 150 160	
121	A C T G C A A G G A T T A A C A G T G A G A A C A T G A A G C T G C C A C T T T	gphkng1815-1.
121	A C T G C A A G G A T T A A C A G T G A G A A C A T G A A G C T G C C A C T T T	gp7b-1.
121	A C T G C A A G G A T T A A C A G T G A G A A C A T G A A G C T G C C A C T T T	gp7c-1.
121	A C T G C A A G G A T T A A C A G T G A G A A C A T G A A G C T G C C A C T T T	gp7d-1.
	T G A T G T T T C C C G T G T G T C T G C T A T G G T T G A A A G A C T G T C A	Majority
	170 180 190 200	
161	T G A T G T T T C C C G T G T G T C T G C T A T G G T T G A A A G A C T G T C A	gphkng1815-1.
161	T G A T G T T T C C C G T G T G T C T G C T A T G G T T G A A A G A C T G T C A	gp7b-1.
161	T G A T G T T T C C C G T G T G T C T G C T A T G G T T G A A A G A C T G T C A	gp7c-1.
161	T G A T G T T T C C C G T G T G T C T G C T A T G G T T G A A A G A C T G T C A	gp7d-1.
	T T G T G C A C C T A C T T G G A A G G A C A A A A C T G C C A T C A G T G A A	Majority
	210 220 230 240	
201	T T G T G C A C C T A C T T G G A A G G A C A A A A C T G C C A T C A G T G A A	gphkng1815-1.
201	T T G T G C A C C T A C T T G G A A G G A C A A A A C T G C C A T C A G T G A A	gp7b-1.
201	T T G T G C A C C T A C T T G G A A G G A C A A A A C T G C C A T C A G T G A A	gp7c-1.
201	T T G T G C A C C T A C T T G G A A G G A C A A A A C T G C C A T C A G T G A A	gp7d-1.
	A A C G C G A A C A G T T T T T C T G A G G C T G G G G A G A T A G A C G T A G	Majority
	250 260 270 280	
241	A A C G C G A A C A G T T T T T C T G A G G C T G G G G A G A T A G A C G T A G	gphkng1815-1.
241	A A C G C G A A C A G T T T T T C T G A G G C T G G G G A G A T A G A C G T A G	gp7b-1.
241	A A C G C G A A C A G T T T T T C T G A G G C T G G G G A G A T A G A C G T A G	gp7c-1.
241	A A C G C G A A C A G T T T T T C T G A G G C T G G G G A G A T A G A C G T A G	gp7d-1.

FIG. 14A

```
    A T G G A G A G G T G A A G A T A G C T T T G A T T G G C A T T A A A C A G A T  Majority
                       290                     300                     310                     320
281 A T G G A G A G G T G A A G A T A G C T T T G A T T G G C A T T A A A C A G A T  gphkng1815-
281 A T G G A G A G G T G A A G A T A G C T T T G A T T G G C A T T A A A C A G A T  gp7b-1.
281 A T G G A G A G G T G A A G A T A G C T T T G A T T G G C A T T A A A C A G A T  gp7c-1.
281 A T G G A G A G G T G A A G A T A G C T T T G A T T G G C A T T A A A C A G A T  gp7d-1.

    G A A A A T C A T G A T G G A A A G G A G A G A G G A A G A A C A C A G C A A A  Majority
                       330                     340                     350                     360
321 G A A A A T C A T G A T G G A A A G G A G A G A G G A A G A A C A C A G C A A A  gphkng1815-
321 G A A A A T C A T G A T G G A A A G G A G A G A G G A A G A A C A C A G C A A A  gp7b-1.
321 G A A A A T C A T G A T G G A A A G G A G A G A G G A A G A A C A C A G C A A A  gp7c-1.
321 G A A A A T C A T G A T G G A A A G G A G A G A G G A A G A A C A C A G C A A A  gp7d-1.

    C T A A T G A A A A C C T T G A A G A A G T G C A A A G A A G A A A A G C A G G  Majority
                       370                     380                     390                     400
361 C T A A T G A A A A C C T T G A A G A A G T G C A A A G A A G A A A A G C A G G  gphkng1815-
361 C T A A T G A A A A C C T T G A A G A A G T G C A A A G A A G A A A A G C A G G  gp7b-1.
361 C T A A T G A A A A C C T T G A A G A A G T G C A A A G A A G A A A A G C A G G  gp7c-1.
361 C T A A T G A A A A C C T T G A A G A A G T G C A A A G A A G A A A A G C A G G  gp7d-1.

    A G G C C C T G A A A C T T A T G A A T G A A G T T C A T G A A C A C C T G G A  Majority
                       410                     420                     430                     440
401 A G G C C C T G A A A C T T A T G A A T G A A G T T C A T G A A C A C C T G G A  gphkng1815-
401 A G G C C C T G A A A C T T A T G A A T G A A G T T C A T G A A C A C C T G G A  gp7b-1.
401 A G G C C C T G A A A C T T A T G A A T G A A G T T C A T G A A C A C C T G G A  gp7c-1.
401 A G G C C C T G A A A C T T A T G A A T G A A G T T C A T G A A C A C C T G G A  gp7d-1.

    G G A G G A A G A A A G C T T A T G C C A G G T T T C T C T G G C A G A T T C C  Majority
                       450                     460                     470                     480
441 G G A G G A A G A A A G C T T A T G C C A G G T T T C T C T G G C A G A T T C C  gphkng1815-
441 G G A G G A A G A A A G C T T A T G C C A G G T T T C T C T G G C A G A T T C C  gp7b-1.
441 G G A G G A A G A A A G C T T A T G C C A G G T T T C T C T G G C A G A T T C C  gp7c-1.
441 G G A G G A A G A A A G C T T A T G C C A G G T T T C T C T G G C A G A T T C C  gp7d-1.

    T G G G A T G A A T G C A G G G C T T G C C T G G A A A G T A A C T G C A T G A  Majority
                       490                     500                     510                     520
481 T G G G A T G A A T G C A G G G C T T G C C T G G A A A G T A A C T G C A T G A  gphkng1815-
481 T G G G A T G A A T G C A G G G C T T G C C T G G A A A G T A A C T G C A T G A  gp7b-1.
481 T G G G A T G A A T G C A G G G C T T G C C T G G A A A G T A A C T G C A T G A  gp7c-1.
481 T G G G A T G A A T G C A G G G C T T G C C T G G A A A G T A A C T G C A T G A  gp7d-1.

    G G T T T G A T A C C A C C T G C C A A C C T G C A T G G T C C T C T G T G A A  Majority
                       530                     540                     550                     560
521 G G T T T G A T A C C A C C T G C C A A C C T G C A T G G T C C T C T G T G A A  gphkng1815-
521 G G T T T G A T A C C A C C T G C C A A C C T G C A T G G T C C T C T G T G A A  gp7b-1.
521 G G T T T G A T A C C A C C T G C C A A C C T G C A T G G T C C T C T G T G A A  gp7c-1.
521 G G T T T G A T A C C A C C T G C C A A C C T G C A T G G T C C T C T G T G A A  gp7d-1.
```

FIG. 14B

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    AAATATGG--------------------------------  Majority
               570       580       590       600
561 AAATATGGTGGAACAGTTTTTCAGGAAGATCTATCAGTTT  gphkng1815-1.
561 AAATATGG--------------------------------  gp7b-1.
561 AAATATGG--------------------------------  gp7c-1.
561 AAATATG---------------------------------  gp7d-1.

    ----------------AAAATGACAGAAGTGGCCCTGTCA  Majority
               610       620       630       640
601 CTGTTTCCTCTCCAGGAAAATGACAGAAGTGGCCCTGTCA  gphkng1815-1.
569 ----------------AAAATGACAGAAGTGGCCCTGTCA  gp7b-1.
569 ----------------------------------------  gp7c-1.
568 ----------------------------------------  gp7d-1.

    GCAAAGGGGTCACTGAGGAAGATGCGCAGGTGTCACACAT  Majority
               650       660       670       680
641 GCAAAGGGGTCACTGAGGAAGATGCGCAGGTGTCACACAT  gphkng1815-1.
593 GCAAAGGGGTCACTGAGGAAGATGCGCAGGTGTCACACAT  gp7b-1.
569 ----------------------------------------  gp7c-1.
568 ----------------------------------------  gp7d-1.

    AGAGCATGTGTTCAGCCAGCTGAGCGCAGATGTGACATCT  Majority
               690       700       710       720
681 AGAGCATGTGTTCAGCCAGCTGAGCGCAGATGTGACATCT  gphkng1815-1.
633 AGAGCATGTGTTCAGCCAGCTGAGCGCAGATGTGACATCT  gp7b-1.
569 ----------------------------------------  gp7c-1.
568 ----------------------------------------  gp7d-1.

    CTCTTCAACAGAAGCCTTTACGTCTTCAAACAGCTGCGGC  Majority
               730       740       750       760
721 CTCTTCAACAGAAGCCTTTACGTCTTCAAACAGCTGCGGC  gphkng1815-1.
673 CTCTTCAACAGAAGCCTTTACGTCTTCAAACAGCTGCGGC  gp7b-1.
569 ----------------------------------------  gp7c-1.
568 ----------------------------------------  gp7d-1.

    GAGAATTTGACCAGGCTTTTCAGTCATATTTCACATCGGG  Majority
               770       780       790       800
761 GAGAATTTGACCAGGCTTTTCAGTCATATTTCACATCGGG  gphkng1815-1.
713 GAGAATTTGACCAGGCTTTTCAGTCATATTTCACATCGGG  gp7b-1.
569 ----------------------------------------  gp7c-1.
568 ----------------------------------------  gp7d-1.

    GACTGACGTTACAGAGCCTTTCTTTTTTCCATCTTTGTCC  Majority
               810       820       830       840
801 GACTGACGTTACAGAGCCTTTCTTTTTTCCATCTTTGTCC  gphkng1815-1.
753 GACTGACGTTACAGAGCCTTTCTTTTTTCCATCTTTGTCC  gp7b-1.
569 ----------------------------------------  gp7c-1.
568 ----------------------------------------  gp7d-1.
```

FIG. 14C

```
     AAGGAGCCAGCCTACAGAGCAGATGCTGAGCCAAGCTGGG Majority
              850       860       870       880
841  AAGGAGCCAGCCTACAGAGCAGATGCTGAGCCAAGCTGGG gphkng1815-1.
793  AAGGAGCCAGCCTACAGAGCAGATGCTGAGCCAAGCTGGG gp7b-1.
569  ----AGCCAGCCTACAGAGCAGATGCTGAGCCAAGCTGGG gp7c-1.
568  ------CCAGCCTACAGAGCAGATGCTGAGCCAAGCTGGG gp7d-1.

     CCATTCCCAATGTCTTCCAGCTGCTCTGCAACTTGAGTTT Majority
              890       900       910       920
881  CCATTCCCAATGTCTTCCAGCTGCTCTGCAACTTGAGTTT gphkng1815-1.
833  CCATTCCCAATGTCTTCCAGCTGCTCTGCAACTTGAGTTT gp7b-1.
605  CCATTCCCAATGTCTTCCAGCTGCTCTGCAACTTGAGTTT gp7c-1.
602  CCATTCCCAATGTCTTCCAGCTGCTCTGCAACTTGAGTTT gp7d-1.

     CTCAGTTTATCAAAGTGTCAGTGAAAAACTCATCACAACC Majority
              930       940       950       960
921  CTCAGTTTATCAAAGTGTCAGTGAAAAACTCATCACAACC gphkng1815-1.
873  CTCAGTTTATCAAAGTGTCAGTGAAAAACTCATCACAACC gp7b-1.
645  CTCAGTTTATCAAAGTGTCAGTGAAAAACTCATCACAACC gp7c-1.
642  CTCAGTTTATCAAAGTGTCAGTGAAAAACTCATCACAACC gp7d-1.

     CTGCGTGCCACAGAGGACCCTCCAAAACAAGACAAAGACT Majority
              970       980       990       1000
961  CTGCGTGCCACAGAGGACCCTCCAAAACAAGACAAAGACT gphkng1815-1.
913  CTGCGTGCCACAGAGGACCCTCCAAAACAAGACAAAGACT gp7b-1.
685  CTGCGTGCCACAGAGGACCCTCCAAAACAAGACAAAGACT gp7c-1.
682  CTGCGTGCCACAGAGGACCCTCCAAAACAAGACAAAGACT gp7d-1.

     CCAACCAGGGAGGCCCGATTTCAAAGATACTACCTGAGCA Majority
              1010      1020      1030      1040
1001 CCAACCAGGGAGGCCCGATTTCAAAGATACTACCTGAGCA gphkng1815-1.
953  CCAACCAGGGAGGCCCGATTTCAAAGATACTACCTGAGCA gp7b-1.
725  CCAACCAGGGAGGCCCGATTTCAAAGATACTACCTGAGCA gp7c-1.
722  CCAACCAGGGAGGCCCGATTTCAAAGATACTACCTGAGCA gp7d-1.

     AGACAGAGGCTCAGATGGGAAACTTGGCCAGAATTTGTCT Majority
              1050      1060      1070      1080
1041 AGACAGAGGCTCAGATGGGAAACTTGGCCAGAATTTGTCT gphkng1815-1.
993  AGACAGAGGCTCAGATGGGAAACTTGGCCAGAATTTGTCT gp7b-1.
765  AGACAGAGGCTCAGATGGGAAACTTGGCCAGAATTTGTCT gp7c-1.
762  AGACAGAGGCTCAGATGGGAAACTTGGCCAGAATTTGTCT gp7d-1.

     GATTGCGTTAATTTTCGCAAGAGATGCCAGAAATGCCAGG Majority
              1090      1100      1110      1120
1081 GATTGCGTTAATTTTCGCAAGAGATGCCAGAAATGCCAGG gphkng1815-1.
1033 GATTGCGTTAATTTTCGCAAGAGATGCCAGAAATGCCAGG gp7b-1.
805  GATTGCGTTAATTTTCGCAAGAGATGCCAGAAATGCCAGG gp7c-1.
802  GATTGCGTTAATTTTCGCAAGAGATGCCAGAAATGCCAGG gp7d-1.
```

FIG. 14D

```
     A T T A T C T A T C T G A T G A C T G C C C T A A T G T G C C T G A A C T A T A  Majority
                         1130                1140                1150                1160
1121 A T T A T C T A T C T G A T G A C T G C C C T A A T G T G C C T G A A C T A T A  gphkng1815-1.
1073 A T T A T C T A T C T G A T G A C T G C C C T A A T G T G C C T G A A C T A T A  gp7b-1.
845  A T T A T C T A T C T G A T G A C T G C C C T A A T G T G C C T G A A C T A T A  gp7c-1.
842  A T T A T C T A T C T G A T G A C T G C C C T A A T G T G C C T G A A C T A T A  gp7d-1.

     C A G A G A A C T C A A T G A G G C C C T C C G A C T G G T C A G T A G A T C C  Majority
                         1170                1180                1190                1200
1161 C A G A G A A C T C A A T G A G G C C C T C C G A C T G G T C A G T A G A T C C  gphkng1815-1.
1113 C A G A G A A C T C A A T G A G G C C C T C C G A C T G G T C A G T A G A T C C  gp7b-1.
885  C A G A G A A C T C A A T G A G G C C C T C C G A C T G G T C A G T A G A T C C  gp7c-1.
882  C A G A G A A C T C A A T G A G G C C C T C C G A C T G G T C A G T A G A T C C  gp7d-1.

     A A T C A G C A A T A C G A C C A G G T G G T G C A G A T G A C C C A G T A T C  Majority
                         1210                1220                1230                1240
1201 A A T C A G C A A T A C G A C C A G G T G G T G C A G A T G A C C C A G T A T C  gphkng1815-1.
1153 A A T C A G C A A T A C G A C C A G G T G G T G C A G A T G A C C C A G T A T C  gp7b-1.
925  A A T C A G C A A T A C G A C C A G G T G G T G C A G A T G A C C C A G T A T C  gp7c-1.
922  A A T C A G C A A T A C G A C C A G G T G G T G C A G A T G A C C C A G T A T C  gp7d-1.

     A C C T G G A A G A C A C C A C G C T T C T G A T G G A G A A G A T G A G A G A  Majority
                         1250                1260                1270                1280
1241 A C C T G G A A G A C A C C A C G C T T C T G A T G G A G A A G A T G A G A G A  gphkng1815-1.
1193 A C C T G G A A G A C A C C A C G C T T C T G A T G G A G A A G A T G A G A G A  gp7b-1.
965  A C C T G G A A G A C A C C A C G C T T C T G A T G G A G A A G A T G A G A G A  gp7c-1.
962  A C C T G G A A G A C A C C A C G C T T C T G A T G G A G A A G A T G A G A G A  gp7d-1.

     G C A G T T T G G C T G G G T T T C T G A A C T G G C A T A C C A G T C C C C A  Majority
                         1290                1300                1310                1320
1281 G C A G T T T G G C T G G G T T T C T G A A C T G G C A T A C C A G T C C C C A  gphkng1815-1.
1233 G C A G T T T G G C T G G G T T T C T G A A C T G G C A T A C C A G T C C C C A  gp7b-1.
1005 G C A G T T T G G C T G G G T T T C T G A A C T G G C A T A C C A G T C C C C A  gp7c-1.
1002 G C A G T T T G G C T G G G T T T C T G A A C T G G C A T A C C A G T C C C C A  gp7d-1.

     G G A G C T G A G G A C A T C T T T A A T C C A G T G A A A G T A A T G G T A G  Majority
                         1330                1340                1350                1360
1321 G G A G C T G A G G A C A T C T T T A A T C C A G T G A A A G T A A T G G T A G  gphkng1815-1.
1273 G G A G C T G A G G A C A T C T T T A A T C C A G T G A A A G T A A T G G T A G  gp7b-1.
1045 G G A G C T G A G G A C A T C T T T A A T C C A G T G A A A G T A A T G G T A G  gp7c-1.
1042 G G A G C T G A G G A C A T C T T T A A T C C A G T G A A A G T A A T G G T A G  gp7d-1.

     C C C T A A G T G C T C A T G A A G G A A A T T C T T C T G A T C A A G A T G A  Majority
                         1370                1380                1390                1400
1361 C C C T A A G T G C T C A T G A A G G A A A T T C T T C T G A T C A A G A T G A  gphkng1815-1.
1313 C C C T A A G T G C T C A T G A A G G A A A T T C T T C T G A T C A A G A T G A  gp7b-1.
1085 C C C T A A G T G C T C A T G A A G G A A A T T C T T C T G A T C A A G A T G A  gp7c-1.
1082 C C C T A A G T G C T C A T G A A G G A A A T T C T T C T G A T C A A G A T G A  gp7d-1.
```

FIG. 14E

```
     CACAGTGGTTCCTTCAAGCCTCCTGCCTTCCTCTAACTTC Majority
              1410      1420      1430      1440
1401 CACAGTGGTTCCTTCAAGCCTCCTGCCTTCCTCTAACTTC gphkng1815-1.
1353 CACAGTGGTTCCTTCAAGCCTCCTGCCTTCCTCTAACTTC gp7b-1.
1125 CACAGTGGTTCCTTCAAGCCTCCTGCCTTCCTCTAACTTC gp7c-1.
1122 CACAGTGGTTCCTTCAAGCCTCCTGCCTTCCTCTAACTTC gp7d-1.

     ACACTCAGCAGCCCTCTTGAAAAGAGTGCTGGCAACGCTA Majority
              1450      1460      1470      1480
1441 ACACTCAGCAGCCCTCTTGAAAAGAGTGCTGGCAACGCTA gphkng1815-1.
1393 ACACTCAGCAGCCCTCTTGAAAAGAGTGCTGGCAACGCTA gp7b-1.
1165 ACACTCAGCAGCCCTCTTGAAAAGAGTGCTGGCAACGCTA gp7c-1.
1162 ACACTCAGCAGCCCTCTTGAAAAGAGTGCTGGCAACGCTA gp7d-1.

     ACTTCATTGATCACGTGGTAGAGAAGGTTCTTCAGCACTT Majority
              1490      1500      1510      1520
1481 ACTTCATTGATCACGTGGTAGAGAAGGTTCTTCAGCACTT gphkng1815-1.
1433 ACTTCATTGATCACGTGGTAGAGAAGGTTCTTCAGCACTT gp7b-1.
1205 ACTTCATTGATCACGTGGTAGAGAAGGTTCTTCAGCACTT gp7c-1.
1202 ACTTCATTGATCACGTGGTAGAGAAGGTTCTTCAGCACTT gp7d-1.

     TAAGGAGCACTTTAAAACTTGGTAAGAAGATTTAGTCCAT Majority
              1530      1540      1550      1560
1521 TAAGGAGCACTTTAAAACTTGGTAAGAAGATTTAGTCCAT gphkng1815-1.
1473 TAAGGAGCACTTTAAAACTTGGTAAGAAGATTTAGTCCAT gp7b-1.
1245 TAAGGAGCACTTTAAAACTTGGTAAGAAGATTTAGTCCAT gp7c-1.
1242 TAAGGAGCACTTTAAAACTTGGTAAGAAGATTTAGTCCAT gp7d-1.

     CCTATAATCAGCAAGAATTACACCTTCGGCCAAGACCTGA Majority
              1570      1580      1590      1600
1561 CCTATAATCAGCAAGAATTACACCTTCGGCCAAGACCTGA gphkng1815-1.
1513 CCTATAATCAGCAAGAATTACACCTTCGGCCAAGACCTGA gp7b-1.
1285 CCTATAATCAGCAAGAATTACACCTTCGGCCAAGACCTGA gp7c-1.
1282 CCTATAATCAGCAAGAATTACACCTTCGGCCAAGACCTGA gp7d-1.

     GAATTCTGAAAATACAAAGCAGGCTAACACAATGAACACA Majority
              1610      1620      1630      1640
1601 GAATTCTGAAAATACAAAGCAGGCTAACACAATGAACACA gphkng1815-1.
1553 GAATTCTGAAAATACAAAGCAGGCTAACACAATGAACACA gp7b-1.
1325 GAATTCTGAAAATACAAAGCAGGCTAACACAATGAACACA gp7c-1.
1322 GAATTCTGAAAATACAAAGCAGGCTAACACAATGAACACA gp7d-1.

     GCTGCATGAAAGTTAGGTATATATTAGGAAGCACTATTGG Majority
              1650      1660      1670      1680
1641 GCTGCATGAAAGTTAGGTATATATTAGGAAGCACTATTGG gphkng1815-1.
1593 GCTGCATGAAAGTTAGGTATATATTAGGAAGCACTATTGG gp7b-1.
1365 GCTGCATGAAAGTTAGGTATATATTAGGAAGCACTATTGG gp7c-1.
1362 GCTGCATGAAAGTTAGGTATATATTAGGAAGCACTATTGG gp7d-1.
```

FIG. 14F

```
     TTTACTTTGTTGAATGGAAGTTTAATAGCTATTCAAATTG Majority
                1690      1700      1710      1720
1681 TTTACTTTGTTGAATGGAAGTTTAATAGCTATTCAAATTG gphkng1815-1.
1633 TTTACTTTGTTGAATGGAAGTTTAATAGCTATTCAAATTG gp7b-1.
1405 TTTACTTTGTTGAATCGAAGTTTAATAGCTATTCAAATTG gp7c-1.
1402 TTTACTTTGTTGAATGGAAGTTTAATAGCTATTCAAATTG gp7d-1.

     AGTTAATATAAAAATTTCTTCCTAAAAAGTAAAATGTACA Majority
                1730      1740      1750      1760
1721 AGTTAATATAAAAATTTCTTCCTAAAAAGTAAAATGTACA gphkng1815-1.
1673 AGTTAATATAAAAATTTCTTCCTAAAAAGTAAAATGTACA gp7b-1.
1445 AGTTAATATAAAAATTTCTTCCTAAAAAGTAAAATGTACA gp7c-1.
1442 AGTTAATATAAAAATTTCTTCCTAAAAAGTAAAATGTACA gp7d-1.

     TATGTAGAATATGATGCATTAGTTCTTTGTATACTAAATA Majority
                1770      1780      1790      1800
1761 TATGTAGAATATGATGCATTAGTTCTTTGTATACTAAATA gphkng1815-1.
1713 TATGTAGAATATGATGCATTAGTTCTTTGTATACTAAATA gp7b-1.
1485 TATGTAGAATATGATGCATTAGTTCTTTGTATACTAAATA gp7c-1.
1482 TATGTAGAATATGATGCATTAGTTCTTTGTATACTAAATA gp7d-1.

     AATACTGAGTCCCCT                          Majority
                1810
1801 AATACTGAGTCCCCT                          gphkng1815-1.
1753 AATACTGAGTCCCCT                          gp7b-1.
1525 AATACTGAGTCCCCT                          gp7c-1.
1522 AATACTGAGTCCCCT                          gp7d-1.
```

FIG. 14G

```
                  1                                                                              80
gphkng1815_aa_    MKLPLLMFPVCLLWLKDCHCAPTWKDKTAISENANSFSEAGEIDVDGEVKIALIGIKQMKIMMERREEEHSKLMKTLKKC
gb7b_aa           MKLPLLMFPVCLLWLKDCHCAPTWKDKTAISENANSFSEAGEIDVDGEVKIALIGIKQMKIMMERREEEHSKLMKTLKKC
gb7c_aa           MKLPLLMFPVCLLWLKDCHCAPTWKDKTAISENANSFSEAGEIDVDGEVKIALIGIKQMKIMMERREEEHSKLMKTLKKC
gb7d_aa           MKLPLLMFPVCLLWLKDCHCAPTWKDKTAISENANSFSEAGEIDVDGEVKIALIGIKQMKIMMERREEEHSKLMKTLKKC
                  81                                                                            160
gphkng1815_aa_    KEEKQEALKLMNEVHEHLEEEESLCQVSLADSWDECRACLESNCMRFDTTCQPAWSSVKNMVEQFFRKIYQFLFPLQEND
gb7b_aa           KEEKQEALKLMNEVHEHLEEEESLCQVSLADSWDECRACLESNCMRFDTTCQPAWSSVKN................MEND
gb7c_aa           KEEKQEALKLMNEVHEHLEEEESLCQVSLADSWDECRACLESNCMRFDTTCQPAWSSVKN....................
gb7d_aa           KEEKQEALKLMNEVHEHLEEEESLCQVSLADSWDECRACLESNCMRFDTTCQPAWSSVKN....................
                  161                                                                           240
gphkng1815_aa_    RSGPVSKGVTEEDAQVSHIEHVFSQLSADVTSLFNRSLYVFKQLRREFDQAFQSYFTSGTDVTEPFFFPSLSKEPAYRAD
gb7b_aa           RSGPVSKGVTEEDAQVSHIEHVFSQLSADVTSLFNRSLYVFKQLRREFDQAFQSYFTSGTDVTEPFFFPSLSKEPAYRAD
gb7c_aa           ........................................................................MEPAYRAD
gb7d_aa           .........................................................................MPAYRAD
                  241                                                                           320
gphkng1815_aa_    AEPSWAIPNVFQLLCNLSFSVYQSVSEKLITTLRATEDPPKQDKDSNQGGPISKILPEQDRGSDGKLGQNLSDCVNFRKR
gb7b_aa           AEPSWAIPNVFQLLCNLSFSVYQSVSEKLITTLRATEDPPKQDKDSNQGGPISKILPEQDRGSDGKLGQNLSDCVNFRKR
gb7c_aa           AEPSWAIPNVFQLLCNLSFSVYQSVSEKLITTLRATEDPPKQDKDSNQGGPISKILPEQDRGSDGKLGQNLSDCVNFRKR
gb7d_aa           AEPSWAIPNVFQLLCNLSFSVYQSVSEKLITTLRATEDPPKQDKDSNQGGPISKILPEQDRGSDGKLGQNLSDCVNFRKR
                  321                                                                           400
gphkng1815_aa_    CQKCQDYLSDDCPNVPELYRELNEALRLVSRSNQQYDQVVQMTQYHLEDTTLLMEKMREQFGWVSELAYQSPGAEDIFNP
gb7b_aa           CQKCQDYLSDDCPNVPELYRELNEALRLVSRSNQQYDQVVQMTQYHLEDTTLLMEKMREQFGWVSELAYQSPGAEDIFNP
gb7c_aa           CQKCQDYLSDDCPNVPELYRELNEALRLVSRSNQQYDQVVQMTQYHLEDTTLLMEKMREQFGWVSELAYQSPGAEDIFNP
gb7d_aa           CQKCQDYLSDDCPNVPELYRELNEALRLVSRSNQQYDQVVQMTQYHLEDTTLLMEKMREQFGWVSELAYQSPGAEDIFNP
                  401                                                                           466
gphkng1815_aa_    VKVMVALSAHEGNSSDQDDTVVPSSLLPSSNFTLSSPLEKSAGNANFIDHVVEKVLQHFKEHFKTW
gb7b_aa           VKVMVALSAHEGNSSDQDDTVVPSSLLPSSNFTLSSPLEKSAGNANFIDHVVEKVLQHFKEHFKTW
gb7c_aa           VKVMVALSAHEGNSSDQDDTVVPSSLLPSSNFTLSSPLEKSAGNANFIDHVVEKVLQHFKEHFKTW
gb7d_aa           VKVMVALSAHEGNSSDQDDTVVPSSLLPSSNFTLSSPLEKSAGNANFIDHVVEKVLQHFKEHFKTW
```

FIG. 14H

```
         1                                                                              80
bhkng1   ~~~~~~~~~~~~~~~~~~~~~~~~~GCAACCTCGTTGGTGAGAGCCTGCAGTTAGTGTCACGGCGGAAACATGAAGCCGC
bhkng2   ~~~~~~~~~~~~~CAGAAGCTGGTGGCAACCTCGTTGGTGAGAGCCTGCAGTTAGTGTCACGGCGGAAACATGAAGCCGC
bhkng3   GTGAAGGTCCTTACAGAAGCTGGTGGCAACCTCGTTGGTGAGAGCCTGCAGTTAGTGTCACGGCGGAAACATGAAGCCGC
         81                                                                            160
bhkng1   CACTCTTGGTGTTTATTGTGTATCTGCTGCGGCTGAGAGACTGTCAGTGTGCGCCTACAGGGAAGGACCGAACTTCCATC
bhkng2   CACTCTTGGTGTTTATTGTGTATCTGCTGCGGCTGAGAGACTGTCAGTGTGCGCCTACAGGGAAGGACCGAACTTCCATC
bhkng3   CAATCTTGGTGTTTATCGTGTATCTGCTGCAGCTGAGAGACTGTCAGTGTGCGCCTACAGGGAAGGACCGAACTTCCATC
         161                                                                           240
bhkng1   CGTGAAGACCCGAAGGGTTTTTCCAAGGCTGGGGAGATAGACGTAGATGAAGAGGTGAAGAAGGCTTTGATTGGCATGAA
bhkng2   CGTGAAGACCCGAAGGGTTTTTCCAAGGCTGGGGAGATAGACGTAGATGAAGAGGTGAAGAAGGCTTTGATTGGCATGAA
bhkng3   CGTGAAGACCCGAAGGGTTTTTCCAAGGCTGGGGAGATAGACGTAGATGAAGAGGTGAAGAAGGCTTTGATTGGCATGAA
         241                                                                           320
bhkng1   GCAGATGAAAATCCTGATGGAAAGAAGAGAGGAGGAACATAGCAAACTAATGAGAACACTGAAGAAATGCAGAGAAGAAA
bhkng2   GCAGATGAAAATCCTGATGGAAAGAAGAGAGGAGGAACATAGCAAACTAATGAGAACACTGAAGAAATGCAGAGAAGAAA
bhkng3   GCAGATGAAAATCCTGATGGAAAGAAGAGAGGAGGAACATAGCAAACTAATGAGAACCCTGAAGAAATGCAGAGAAGAAA
         321                                                                           400
bhkng1   AGCAGGAGGCCCTGAAGCTTATGAATGAAGTTCAAGAACATCTAGAAGAGGAAGAAAGGCTATGCCAGGTGTCTCTGATG
bhkng2   AGCAGGAGGCCCTGAAGCTTATGAATGAAGTTCAAGAACATCTAGAAGAGGAAGAAAGGCTATGCCAGGTGTCTCTGATG
bhkng3   AGCAGGAGGCCCTGAAGCTTATGAATGAAGTTCAAGAACATCTAGAAGAGGAAGAAAGGCTATGCCAGGTGTCTCTGATG
         401                                                                           480
bhkng1   GGTTCCTGGGACGAATGCAAATCTTGCCTGGAAAGTGACTGCATGAGATTTTATACAACCTGCCAAAGCAGTTGGTCCTC
bhkng2   GGTTCCTGGGACGAATGCAAATCTTGCCTGGAAAGTGACTGCATGAGATTTTATACAACCTGCCAAAGCAGTTGGTCCTC
bhkng3   GGTTCCTGGGACGAATGCAAATCTTGCCTGGAAAGTGACTGCATGAGATTTTATACAACCTGCCAAAGCAGTTGGTCCTC
         481                                                                           560
bhkng1   TATGAAATCCACGATTGAACGGGTTTTCCGGAAGATATATCAGTTTCTCTTTCCTTTCCATGAAGACGATGAAAAAGAGC
bhkng2   TATGAAATCCACGATTGAACGGGTTTTCCGGAAGATATATCAGTTTCTCTTTCCTTTCCATGAAGACGATGAAAAAGAGC
bhkng3   TATGAAATCCACGATTGAACGGGTTTTCCGGAAGATATATCAGTTTCTCTTTCCTTTCCATGAAGACGATGAAAAAGAGC
         561                                                                           640
bhkng1   TTCCTGTTGGTGAGAAGTTCACTGAGGAAGATGTACAGCTGATGCAGATAGAGAATGTGTTCAGCCAGCTGACCGTGGAT
bhkng2   TTCCTGTTGGTGAGAAGTTCACTGAGGAAGATGTACAGCTGATGCAGATAGAGAATGTGTTCAGCCAGCTGACCGTGGAT
bhkng3   TTCCTGTTGGTGAGAAGTTCACTGAGGAAGATGTACAGCTGATGCAGATAGAGAATGTGTTCAGCCAGCTGACCGTGGAC
         641                                                                           720
bhkng1   GTGGGATTTCTCTATAACATGAGCTTTCACGTCTTCAAACAGATGCAGCAAGAATTTGACCTGGCTTTTCAATCATACTT
bhkng2   GTGGGATTTCTCTATAACATGAGCTTTCACGTCTTCAAACAGATGCAGCAAGAATTTGACCTGGCTTTTCAATCATACTT
bhkng3   GTGGGATTTCTCTATAACATGAGCTTTCACGTCTTCAAACAGATGCAGCAAGAATTTGACCTGGCTTTTCAATCATACTT

         721                                                                           800
bhkng1   TATGTCAGACACAGACTCCATGGAGCCTTACTTTTTTCCAGCTTTTTCCAAAGAGCCAGCAAAAAAAGCACATCCTATGC
bhkng2   TATGTCAGACACAGACTCCATGGAGCCTTACTTTTTTCCAGCTTTTTCCAAAGAGCCAGCAAAAAAAGCACATCCTATGC
bhkng3   TATGTCAGACACAGACTCCATGGAGCCTTACTTTTTTCCAGCTTTTTCCAAAGAGCCAGCAAAAAAAGCACATCCTATGC
         801                                                                           880
bhkng1   AGAGTTGGGACATTCCCAGCTTCTTCCAGCTGTTTTGTAATTTCAGCCTCTCTGTTTATCAAAGTGTCAGCGCAACAGTT
bhkng2   AGAGTTGGGACATTCCCAGCTTCTTCCAGCTGTTTTGTAATTTCAGCCTCTCTGTTTATCAAAGTGTCAGCGCAACAGTT
bhkng3   AGAGTTGGGACATTCCCAGCTTCTTCCAGCTGTTTTGTAATTTCAGCCTCTCTGTTTATCAAAGTGTCAGCGCAACAGTT
```

FIG. 15A

```
          881                                                                           960
bhkng1    ACAGAGATGCTGAAGGCCATTGAGGACTTATCCAAACAAGACAAAGATTCTGCCCACGGTGGACCGAGTTCCACGACGTG
bhkng2    ACAGAGATGCTGAAGGCCATTGAGGACTTATCCAAACAAGACAAAGATTCTGCCCACGGTGGACCGAGTTCCACGACGTG
bhkng3    ACAGAGATGCTGAAGGCCATTGAGGACTTATCCAAACAAGACAAAGATTCTGCCCACGGTGGACCGAGTTCCACGACGTG
          961                                                                          1040
bhkng1    GCCTGTGCGGGGCAGAGGGCTGTGTGGAGAACCTGGCCAGAACTCGTCCGAATGTCTCCAATTTCATGCAAGATGCCAGA
bhkng2    GCCTGTGCGGGGCAGAGGGCTGTGTGGAGAACCTGGCCAGAACTCGTCCGAATGTCTCCAATTTCATGCAAGATGCCAGA
bhkng3    GCCTGTGCGGGGCAGAGGGCTGTGTGGAGAACCTGGCCAGAACTCGTCCGAATGTCTCCAATTTCATGCAAGATGCCAGA
          1041                                                                         1120
bhkng1    AATGTCAGGATTACCTATGGGCAGACTGCCCTGCTGTTCCTGAACTATACACAAAGGCGGATGAGGCCCTTGAGTTGGTC
bhkng2    AATGTCAGGATTACCTATGGGCAGACTGCCCTGCTGTTCCTGAACTATACACAAAGGCGGATGAGGCCCTTGAGTTGGTC
bhkng3    AATGTCAGGATTACCTATGGGCAGACTGCCCTGCTGTTCCTGAACTATACACAAAGGCGGATGAGGCCCTTGAGTTGGTC
          1121                                                                         1200
bhkng1    AACATATCCAATCAGCAGTATGCCCAGGTACTCCAGATGACCCAGCATCACTTGGAGGACACCACGTATCTGATGGAGAA
bhkng2    AACATATCCAATCAGCAGTATGCCCAGGTACTCCAGATGACCCAGCATCACTTGGAGGACACCACGTATCTGATGGAGAA
bhkng3    AACATATCCAATCAGCAGTATGCCCAGGTACTCCAGATGACCCAGCATCACTTGGAGGACACCACGTATCTGATGGAGAA

          1201                                                                         1280
bhkng1    GATGAGAGAGCAGTTTGGTTGGGTAACAGAGCTGGCCAGCCAGACCCCAGGAAGCGAGAACATCTTCAGTTTCATAAAGG
bhkng2    GATGAGAGAGCAGTTTGGTTGGGTAACAGAGCTGGCCAGCCAGACCCCAGGAAGCGAGAACATCTTCAGTTTCATAAAGG
bhkng3    GATGAGAGAGCAGTTTGGTTGGGTAACAGAGCTGGCCAGCCAGACCCCAGGAAGCGAGAACATCTTCAGTTTCATAAAGG
          1281                                                                         1360
bhkng1    TAGTTCCAGGTGTTCACGAAGGAAATTTCTCCAAACAAGATGAAAAGATGATAGACATAAGCATTCTGCCTTCCTCTAAT
bhkng2    TAGTTCCAGGTGTTCACGAAGGAAATTTCTCCAAACAAGATGAAAAGATGATAGACATAAGCATTCTGCCTTCCTCTAAT
bhkng3    TAGTTCCAGGTGTTCACGAAGGAAATTTCTCCAAACAAGATGAAAAGATGATAGACATAAGCATTCTGCCTTCCTCTAAT
          1361                                                                         1440
bhkng1    TTCACACTCACCATCCCTCTTGAAGAAAGTGCTGAGAGTTCCGACTTCATTAGCTACATGCTGGCCAAAGCTGTACAGCA
bhkng2    TTCACACTCACCATCCCTCTTGAAGAAAGTGCTGAGAGTTCCGACTTCATTAGCTACATGCTGGCCAAAGCTGTACAGCA
bhkng3    TTCACACTCACCATCCCTCTTGAAGAAAGTGCTGAGAGTTCCGACTTCATTAGCTACATGCTGGCCAAAGCTGTACAGCA

          1441                                                                         1520
bhkng1    TTTTAAGGAACATTTTAAATCTTGGTAAGCAGAGTATTTGATTAGGGACGTTTGCTGATAGGAATAGATGGTTCTTAAAA
bhkng2    TTTTAAGGAACATTTTAAATCTTGGTAAGCAGAGTATTTGATTAGGGACGTTTGCTGATAGGAATAGATGGTTCTTAAAA
bhkng3    TTTTAAGGAACATTTTAAATCTTGGTAAGCAGAGTATTTGATTAGGGACGTTTGCTGATAGGAATAGATGGTTCTTAAAA
          1521                                                                         1600
bhkng1    GGGAAAAATGACAAAACTAGCTTTTGAATACCTTGAAAACGTATTCAACCTCATTAATAATCAAAGGCATGAAAACTAAG
bhkng2    GGGAAAAATGACAAAACTAGCTTTTGAATACCTTGAAAACGTATTCAACCTCATTAATAATCAAAGGCATGAAAACTAAG
bhkng3    GGGAAAAATGACAAAACTAGCTTTTGAATACCTTGAAAACGTATTCAACCTCATTAATAATCAAAGGCATGAAAACTAAG
          1601                                                                         1680
bhkng1    ACAAGTTAGCAGTTTTTACCTATTGAATTTTCAAATTAAAAAAAAAAATCCTGATAGAATGCAATGAAATGAGAATTCTT
bhkng2    ACAAGTTAGCAGTTTTTACCTATTGAATTTTCAAATTAAAAAAAAAAATCCTGATAGAATGCAATGAAATGAGAATTCTT
bhkng3    ACAAGTTAGCAGTTTTTACCTATTGAATTTTCAAATTAAAAAAAAAA.TCCTGATAGAATGCAATGAAATGAGAATTCTT
          1681                                                                         1760
bhkng1    ATATGTGATTGCCAGAAACAAACTGGTTTTGTCTTTTTGAAAAGTTATTCAATTATACATATCAAGAGTCATCAAATTTC
bhkng2    ATATGTGATTGCCAGAAACAAACTGGTTTTGTCTTTTTGAAAAGTTATTCAATTATACATATCAAGAGTCATCAAATTTC
bhkng3    ATATGTGATTGCCAGAAACAAACTGGTTTTGTCTTTTTGAAAAGTTATTCAATTATACATATCAAGAGTCATCAAATTTC
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FIG. 15B

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        1761                                                                            1840
bhkng1  TTTTTAATATAATAATTCCACTTCTGGAATCAATCCAAAGGAGTAAATCTAAAATTGAATTGAAGTTCCCACCCCAAGAT
bhkng2  TTTTTAATATAATAATTCCACTTCTGGAATCAATCCAAAGGAGTAAATCTAAAATTGAATTGAAGTTCCCACCCCAAGAT
bhkng3  TTTTTAATATAATAATTCCACTTCTGGAATCAATCCAAAGGAGTAAATCTAAAATTGAATTGAAGTTCCCACCCCAAGAT
        1841                                                                            1920
bhkng1  CAATATTTGCAAATTATTTAAAATAGTAAACTGTTAAAAACTGAATGTCATCTGAATGTCTAAAAACCAGAAATGGTTAA
bhkng2  CAATATTTGCAAATTATTTAAAATAGTAAACTGTTAAAAACTGAATGTCATCTGAATGTCTAAAAACCAGAAATGGTTAA
bhkng3  CAATATTTGCAAATTATTTAAAATAGTAAACTGTTAAAAACTGAATGTCATCTGAATGTCTAAAAACCAGAAATGGTTAA
        1921                                                                            2000
bhkng1  AAGCTGTGGCTAAATATGCTCCAAATATCTTATAAAACCATTAAAAATATTTATAAAATTTAAATCATGACATGACATCT
bhkng2  AAGCTGTGGCTAAATATGCTCCAAATATCTTATAAAACCATTAAAAATATTTATAAAATTTAAATCATGACATGACATCT
bhkng3  AAGCTGTGGCTAAATATGCTCCAAATATCTTATAAAACCATTAAAAATATTTATAAAATTTAAATCATGACATGACATCT
        2001                                                                            2080
bhkng1  GCTGGAACAAGAGTTTATTCTAAGCCTATCTATAAGGCAAATATTATTATTACTATCTTCCAGAAAAGAAACTTGAGACT
bhkng2  GCTGGAACAAGAGTTTATTCTAAGCCTATCTATAAGGCAAATATTATTATTACTATCTTCCAGAAAAGAAACTTGAGACT
bhkng3  GCTGGAACAAGAGTTTATTCTAAGCCTATCTATAAGGCAAATATTATTATTACTATCTTCCAGAAAAGAAACTTGAGACT
        2081                                                                            2160
bhkng1  CAGGGTCCAAGTGTTAGTTGCTCAGTCATGTCTGACTCTTTGGGACCCCTTGGACTGTAGCCCACCAGGCTCCTCTGTCC
bhkng2  CAGGGTCCAAGTGTTAGTTGCTCAGTCATGTCTGACTCTTTGAGACCCCTTGGACTGTGGCCCACCAGGCTCCTCTGTCC
bhkng3  CAGGGTCCAAGTGTTAGTTGCTCAGTCATGTCTGACTCTTTGAGACCCCTTGGACTGTAGCCCACCAGGCTCCTCTGTCC

        2161                                                                            2240
bhkng1  GTGGGATTCTTCAGACAGGAATACTGGGGCAGGTTGCTATTTCCTTCTCCAGGAAATCTTCCCTATCCAGGGATGGAACC
bhkng2  ATGGGATTCTTCAGACAAGAATACTGGAGCAGGTTGCTATTTCCTTCTCCAGGAAATCTTCCCTATCCAGGGATGGAACC
bhkng3  ATGGGATTCTTCAGACAAGAATACTGGAGCAGGTTGCTATTTCCTTCTCCAGGAAATCTTCCCTATCCAGGGATGGAACC
        2241                                                                            2320
bhkng1  CAGGTCTCCTGCATTGCAGGTAGATGCTTTACTATCTGAGCAACCAAATGAATTACTCAAGTCAGTAGGGGGTAGAGGCA
bhkng2  CAGGTCTCCTGCATTGCAGGTAGATGCTTTACTATCTGAGCAACCAAATGAATTACTCAAGTCAGTAGGGGGTAGAGGCA
bhkng3  CAGGTCTCCTGCATTGCAGGTAGATGCTTTACTATCTGAGCAACCAAATGAATTACTCAAGTCAGTAGGGGGTAGAGGCA
        2321                                                                            2400
bhkng1  AATTTTAACTTAGTTTTCTCTGAATCATAATTGCCACATTAAACTGGTTCCTGTTGGGACATTTGGTTGAAAAAAAATAAA
bhkng2  AATTTTAACTTAGTTTTCTCTGAATCATAATTGCCACATTAAACTGGTTCCTGTTGGGACATTTGGTTGAAAAAAAATAAA
bhkng3  AATTTTAACTTAGTTTTCTCTGAATCATAATTGCCACATTAAACTGGTTCCTGTTGGGACATTTGGTTGAAAAAAAATAAA

        2401                                                                            2480
bhkng1  GTGAAAAAATGAGTATAAAACTCTATAAATGTAATGATCAAAACGAAAAAAAAATCTACAATCTGCATTAAAAAATAAAAAGG
bhkng2  GTGAAAAAATGAGTATAAAACTCTATAAATGTAATGATCAAAACGAAAAAAAAATCTACAATCTGCATTAAAAAATAAAAAGG
bhkng3  GTGAAAAAATGAGTATAAAACTCTATAAATGTAATGATCAAAACGAAAAAAAAATCTACAATCTGCATTAAAAAATAAAAAGG
        2481                                                                            2560
bhkng1  GTTGGCAGG.......................................................................
bhkng2  GTTGGCAGGAATTACGGTTGGAAATGGATGATTTTTTTAACCTTTTCATCTTTTGATATTTTACAATTTTCTATAATGA
bhkng3  GTTGGCAGG.......................................................................
        2561                                                                            2640
bhkng1  ................................................................................
bhkng2  ATAAATAATTTTGAGATTTCAAATTAGAAGATATGTTGCTAAAATAGCTAGGTAAATGTAGATTGAACACTGTATCAATG
bhkng3  ................................................................................
        2641                                                                            2720
bhkng1  ................................................................................
bhkng2  TGTTCTCATCTTTAAACTTTAGTATAAGTACTTCTATTCCATGGTAATCCTACAGTAAGACGAAATGTAAATCTGTTCGG
bhkng3  ................................................................................
        2721                                                                            2800
bhkng1  ................................................................................
bhkng2  TCTACAGGAAAAACAACTAAATGACATTTCAGACGTACATTACCATCTCTGTTAGGATAATCTTCTGAATTAATGGCACA
bhkng3  ................................................................................
        2801                                                                            2880
bhkng1  ................................................................................
bhkng2  ATTAGAACTGTACATAGTATTCTCCTTTGGTAAAATGGTCAATCTTAAAGAAGCATTAAATGTTAATTCTAAGTTATTAC
bhkng3  ................................................................................
        2881                                                                            2960
bhkng1  ................................................................................
bhkng2  TCATAAGGGACCTTGTAGGTAGGTCCCTATCAATGTATAATTAAGCTGGGTATTTCTAGATTCGCTGCCTCTCCCTTTAT
bhkng3  ................................................................................
        2961                                                                            3029
bhkng1  .....................................................................
bhkng2  CTCTGAATGTTGGAGAGGTTGTTGGTCATCAATCAACCAATATCTTTTTAGCATCTTCTAAGTGAAGGC
bhkng3  .....................................................................
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FIG. 15C

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                   1                                                                            80
hmhkng_aa          MKIKAEKNEGPSRSWWQLHWGDIANNSGNMKPPLLVFIVCLLWLKDSHCAPTWKDKTAISENLKSFSEVGEIDADEEVKK
bhkng1_aa          -----------------------------MKPPLLVFIVYLLRLRDCQCAPTGKDRTSIREDPKGFSKAGEIDVDEEVKK
gphkng1815_aa_     -----------------------------MKLPLLMFPVCLLWLKDCHCAPTWKDKTAISENANSFSEAGEIDVDGEVKI
                   81                                                                          160
hmhkng_aa          ALTGIKQMKIMMERKEKEHTNLMSTLKKCREEKQEALKLLNEVQEHLEEEERLCRESLADSWGECRSCLENNCMRIYTTC
bhkng1_aa          ALIGMKQMKILMERREEEHSKLMRTLKKCREEKQEALKLMNEVQEHLEEEERLCQVSLMGSWDECKSCLESDCMRFYTTC
gphkng1815_aa_     ALIGIKQMKIMMERREEEHSKLMKTLKKCKEEKQEALKLMNEVHEHLEEEESLCQVSLADSWDECRACLESNCMRFDTTC
                   161                                                                         240
hmhkng_aa          QPSWSSVKNKIERFFRKIYQFLFPFHEDNEKDLPISEKLIEEDAQLTQMEDVFSQLTVDVNSLFNRSFNVFRQMQQEFDQ
bhkng1_aa          QSSWSSMKSTIERVFRKIYQFLFPFHEDDEKELPVGEKFTEEDVQLMQIENVFSQLTVDVGFLYNMSFHVFKQMQQEFDL
gphkng1815_aa_     QPAWSSVKNMVEQFFRKIYQFLFPLQE.NDRSGPVSKGVTEEDAQVSHIEHVFSQLSADVTSLFNRSLYVFKQLRREFDQ
                   241                                                                         320
hmhkng_aa          TFQSHFISDTDLTEPYFFPAFSKEPMTKADLEQCWDIPNFFQLFCNFSVSIYESVSETITKMLKAIEDLPKQDKAPDHGG
bhkng1_aa          AFQSYFMSDTDSMEPYFFPAFSKEPAKKAHPMQSWDIPSFFQLFCNFSLSVYQSVSATVTEMLKAIEDLSKQDKDSAHGG
gphkng1815_aa_     AFQSYFTSGTDVTEPFFFPSLSKEPAYRADAEPSWAIPNVFQLLCNLSFSVYQSVSEKLITTLRATEDPPKQDKDSNQGG
                   321                                                                         400
hmhkng_aa          LISKMLPGQDRGLCGELDQNLSRCFKFHEKCQKCQAHLSEDCPDVPALHTELDEAIRLVNVSNQQYGQILQMTRKHLEDT
bhkng1_aa          PSSTTWPVRGRGLCGEPGQNSSECLQFHARCQKCQDYLWADCPAVPELYTKADEALELVNISNQQYAQVLQMTQHHLEDT
gphkng1815_aa_     PISKILPEQDRGSDGKLGQNLSDCVNFRKRCQKCQDYLSDDCPNVPELYRELNEALRLVSRSNQQYDQVVQMTQYHLEDT
                   401                                                                         480
hmhkng_aa          AYLVEKMRGQFGWVSELANQAPETEIIFNSIQVVPRI..HEGNISKQDETMMTDLSILPSSNFTLKIPLEESAESSNFIG
bhkng1_aa          TYLMEKMREQFGWVTELASQTPGSENIFSFIKVVPGV..HEGNFSKQDE.KMIDISILPSSNFTLTIPLEESAESSDFIS
gphkng1815_aa_     TLLMEKMREQFGWVSELAYQSPGAEDIFNPVKVMVALSAHEGNSSDQDD.TVVPSSLLPSSNFTLSSPLEKSAGNANFID
                   481                                                                         497
hmhkng_aa          YVVAKALQHFKEHFKTW
bhkng1_aa          YMLAKAVQHFKEHFKSW
gphkng1815_aa_     HVVEKVLQHFKEHFKTW
```

FIG. 16

```
matureHKNG          ---------------------------------------------------APTWKDKTAIS
HKNG1-V1-IPF3       ------------------MRTWDYSNSGNMKPPLLVFIVCLLWLKDSHCAPTWKDKTAIS
HKNG1/1-V1-IPF2     -------------------------------MKPPLLVFIVCLLWLKDSHCAPTWKDKTAIS
HKNG1-IPF1          MKIKAEKNEGPSRSWWQLHWGDIANNSGNMKPPLLVFIVCLLWLKDSHCAPTWKDKTAIS
                                                                     ***********

matureHKNG          ENLKSFSEVGEIDADEEVKKALTGIKQMKIMMERKEKEHTNLMSTLKKCREEKQEALKLL
HKNG1-V1-IPF3       ENLKSFSEVGEIDADEEVKKALTGIKQMKIMMERKEKEHTNLMSTLKKCREEKQEALKLL
HKNG1/1-V1-IPF2     ENLKSFSEVGEIDADEEVKKALTGIKQMKIMMERKEKEHTNLMSTLKKCREEKQEALKLL
HKNG1-IPF1          ENLKSFSEVGEIDADEEVKKALTGIKQMKIMMERKEKEHTNLMSTLKKCREEKQEALKLL
                    ************************************************************

matureHKNG          NEVQEHLEEEERLCRESLADSWGECRSCLENNCMRIYTTCQPSWSSVKNKIERFFRKIYQ
HKNG1-V1-IPF3       NEVQEHLEEEERLCRESLADSWGECRSCLENNCMRIYTTCQPSWSSVKNKIERFFRKIYQ
HKNG1/1-V1-IPF2     NEVQEHLEEEERLCRESLADSWGECRSCLENNCMRIYTTCQPSWSSVKNKIERFFRKIYQ
HKNG1-IPF1          NEVQEHLEEEERLCRESLADSWGECRSCLENNCMRIYTTCQPSWSSVKNKIERFFRKIYQ
                    ************************************************************

matureHKNG          FLFPFHEDNEKDLPISEKLIEEDAQLTQMEDVFSQLTVDVNSLFNRSFNVFRQMQQEFDQ
HKNG1-V1-IPF3       FLFPFHEDNEKDLPISEKLIEEDAQLTQMEDVFSQLTVDVNSLFNRSFNVFRQMQQEFDQ
HKNG1/1-V1-IPF2     FLFPFHEDNEKDLPISEKLIEEDAQLTQMEDVFSQLTVDVNSLFNRSFNVFRQMQQEFDQ
HKNG1-IPF1          FLFPFHEDNEKDLPISEKLIEEDAQLTQMEDVFSQLTVDVNSLFNRSFNVFRQMQQEFDQ
                    ************************************************************

matureHKNG          TFQSHFISDTDLTEPYFFPAFSKEPMTKADLEQCWDIPNFFQLFCNFSVSIYESVSETIT
HKNG1-V1-IPF3       TFQSHFISDTDLTEPYFFPAFSKEPMTKADLEQCWDIPNFFQLFCNFSVSIYESVSETIT
HKNG1/1-V1-IPF2     TFQSHFISDTDLTEPYFFPAFSKEPMTKADLEQCWDIPNFFQLFCNFSVSIYESVSETIT
HKNG1-IPF1          TFQSHFISDTDLTEPYFFPAFSKEPMTKADLEQCWDIPNFFQLFCNFSVSIYESVSETIT
                    ************************************************************

matureHKNG          KMLKAIEDLPKQDKAPDHGGLISKMLPGQDRGLCGELDQNLSRCFKFHEKCQKCQAHLSE
HKNG1-V1-IPF3       KMLKAIEDLPKQDKAPDHGGLISKMLPGQDRGLCGELDQNLSRCFKFHEKCQKCQAHLSE
HKNG1/1-V1-IPF2     KMLKAIEDLPKQDKAPDHGGLISKMLPGQDRGLCGELDQNLSRCFKFHEKCQKCQAHLSE
HKNG1-IPF1          KMLKAIEDLPKQDKAPDHGGLISKMLPGQDRGLCGELDQNLSRCFKFHEKCQKCQAHLSE
                    ************************************************************

matureHKNG          DCPDVPALHTELDEAIRLVNVSNQQYGQILQMTRKHLEDTAYLVEKMRGQFGWVSELANQ
HKNG1-V1-IPF3       DCPDVPALHTELDEAIRLVNVSNQQYGQILQMTRKHLEDTAYLVEKMRGQFGWVSELANQ
HKNG1/1-V1-IPF2     DCPDVPALHTELDEAIRLVNVSNQQYGQILQMTRKHLEDTAYLVEKMRGQFGWVSELANQ
HKNG1-IPF1          DCPDVPALHTELDEAIRLVNVSNQQYGQILQMTRKHLEDTAYLVEKMRGQFGWVSELANQ
                    ************************************************************

matureHKNG          APETEIIFNSIQVVPRIHEGNISKQDETMMTDLSILPSSNFTLKIPLEESAESSNFIGYV
HKNG1-V1-IPF3       APETEIIFNSIQVVPRIHEGNISKQDETMMTDLSILPSSNFTLKIPLEESAESSNFIGYV
HKNG1/1-V1-IPF2     APETEIIFNSIQVVPRIHEGNISKQDETMMTDLSILPSSNFTLKIPLEESAESSNFIGYV
HKNG1-IPF1          APETEIIFNSIQVVPRIHEGNISKQDETMMTDLSILPSSNFTLKIPLEESAESSNFIGYV
                    ************************************************************

matureHKNG          VAKALQHFKEHFKTW
HKNG1-V1-IPF3       VAKALQHFKEHFKTW
HKNG1/1-V1-IPF2     VAKALQHFKEHFKTW
HKNG1-IPF1          VAKALQHFKEHFKTW
                    ***************
```

FIG. 17

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 R   H   L   Q   A   R   A   A   G   L   V   S   T   L   E   V   A   D   T     19
TG CGT CAC CTG CAG GCC CGG GCC GCG GGG TTG GTT TCC ACC CTG GAG GTT GCT GAC ACC   57

 L   C   P   R   L   T   S   S   R   W   H   R   R   L   Q   G   A   A   L   K     39
CTG TGC CCT CGG CTG ACT TCC AGC CGG TGG CAC AGA CGC CTC CAG GGG GCA GCA CTC AAG  117

 R   I   L   G   M   T   E   L   R   P   S   L   L   P   G   W   S   S   V   A     59
CGC ATC TTA GGA ATG ACA GAG TTG CGT CCC TCT CTG TTG CCA GGC TGG AGT TCA GTG GCA  177

 C   S   *   L   T   E   A   S   N   S   W   V   Q   V   T   L   P   P   Q   P     79
TGT TCT TAG CTC ACT GAA GCC TCA AAT TCC TGG GTT CAA GTG ACC CTC CCA CCT CAG CCC  237

 H   E   D   L   G   L   Q   D   T   A   K   S   L   T   R   M   K   I   K   A     99
CAT GAG GAC CTG GGA CTA CAG GAC ACA GCT AAA TCC CTG ACA CGG ATG AAA ATT AAA GCA  297

 E   K   N   E   G   P   S   R   S   W   W   Q   L   H   W   G   D   I   A   N    119
GAG AAA AAC GAA GGT CCT TCC AGA AGC TGG TGG CAA CTT CAC TGG GGA GAT ATT GCA AAT  357

 N   S   G   N   M   K   P   P   L   L   V   F   I   V   C   L   L   W   L   K    139
AAC AGC GGG AAC ATG AAG CCG CCA CTC TTG GTG TTT ATT GTG TGT CTG CTG TGG TTG AAA  417

 D   S   H   C   A   P   T   W   K   D   K   T   A   I   S   E   N   L   K   S    159
GAC AGT CAC TGC GCA CCC ACT TGG AAG GAC AAA ACT GCT ATC AGT GAA AAC CTG AAG AGT  477

 F   S   E   V   G   E   I   D   A   D   E   E   V   K   K   A   L   T   G   I    179
TTT TCT GAG GTG GGG GAG ATA GAT GCA GAT GAA GAG GTG AAG AAG GCT TTG ACT GGT ATT  537

 K   Q   M   K   I   M   M   E   R   K   E   K   E   H   T   N   L   M   S   T    199
AAG CAA ATG AAA ATC ATG ATG GAA AGA AAA GAG AAG GAA CAC ACC AAT CTA ATG AGC ACC  597

 L   K   K   C   R   E   E   K   Q   E   A   L   K   L   L   N   E   V   Q   E    219
CTG AAG AAA TGC AGA GAA GAA AAG CAG GAG GCC CTG AAA CTT CTG AAT GAA GTT CAA GAA  657

 H   L   E   E   E   E   R   L   C   R   E   S   L   A   D   S   W   G   E   C    239
CAT CTG GAG GAA GAA GAA AGG CTA TGC CGG GAG TCT TTG GCA GAT TCC TGG GGT GAA TGC  717

 R   S   C   L   E   N   N   C   M   R   I   Y   T   T   C   Q   P   S   W   S    259
AGG TCT TGC CTG GAA AAT AAC TGC ATG AGA ATT TAT ACA ACC TGC CAA CCT AGC TGG TCC  777

 S   V   K   N   K   L   L   T   T   E   A   *   F   Q   R   C   Y   L   G   R    279
TCT GTG AAA AAT AAG CTC CTG ACC ACG GAG GCC TGA TTT CAA AGA TGT TAC CTG GGC AGG  837

 T   E   D   C   V   G   N   L   T   R   I   C   Q   D   V   S   N   F   M   K    299
ACA GAG GAC TGT GTG GGG AAC TTG ACC AGA ATT TGT CAA GAT GTT TCA AAT TTC ATG AAA  897

 N   A   K   N   V   R   L   T   Y   L   K   T   V   L   M   Y   L   L   C   T    319
AAT GCC AAA AAT GTC AGG CTC ACC TAT CTG AAG ACT GTC CTG ATG TAC CTG CTC TGC ACA  957

 Q   N   *   T   R   R   S   G   W   S   M   Y   P   I   S   S   M   A   R   F    339
CAG AAT TAG ACG AGG CGA TCA GGT TGG TCA ATG TAT CCA ATC AGC AGT ATG GCC AGA TTC 1017

 S   R   *   P   G   S   T   W   R   T   P   P   I   W   W   R   R   *   E   G    359
TCC AGA TGA CCC GGA AGC ACT TGG AGG ACA CCG CCT ATC TGG TGG AGA AGA TGA GAG GGC 1077

 N   L   A   G   C   L   N   W   Q   T   R   P   Q   K   Q   R   S   S   L   I    379
AAT TTG GCT GGG TGT CTG AAC TGG CAA ACC AGG CCC CAG AAA CAG AGA TCA TCT TTA ATT 1137

 Q   Y   R   *   F   Q   G   F   M   K   E   I   F   P   N   K   M   K   Q   *    399
CAA TAC AGG TAG TTC CAA GGA TTC ATG AAG GAA ATA TTT CCA AAC AAG ATG AAA CAA TGA 1197

 *   Q   T   *   A   F   C   L   P   L   I   S   H   S   R   S   L   L   K   K    419
TGA CAG ACT TAA GCA TTC TGC CTT CCT CTA ATT TCA CAC TCA AGA TCC CTC TTG AAG AAA 1257

 V   L   R   V   L   T   S   L   A   T   *   W   Q   K   L   Y   S   I   L   R    439
GTG CTG AGA GTT CTA ACT TCA TTG GCT ACG TAG TGG CAA AAG CTC TAC AGC ATT TTA AGG 1317
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FIG. 18A

```
N   I   L   K   P   G   K   K   I   *   C   I   L   Y   P   V   S   R   I   I    459
AAC ATT TTA AAA CCT GGT AAG AAG ATC TAA TGC ATC CTA TAT CCA GTA AGT AGA ATT ATC  1377

S   S   S   G   T   W   K   S   *   N   K   K   G   *   C   N   K   H   S   C    479
TCT TCA TCT GGG ACC TGG AAA TCC TGA AAT AAA AAA GGA TAA TGC AAT AAA CAC AGT TGC  1437

R   K   V   C   *   L   Y   T   M   K   Y   S   *   F   T   Y   V   E   W   L    499
AGG AAA GTA TGT TAG CTA TAT ACT ATG AAG TAC TCT TAG TTT ACT TAT GTT GAA TGG CTT  1497

S   Y   *   Y   S   N   *   V   K   M   K   I   P   P   *   K   I   K   R   N    519
AGC TAT TAA TAC TCA AAT TGA GTT AAA ATG AAA ATT CCT CCT TAA AAA ATC AAA CGT AAT  1557

M   Y   Y   I   S   W   Y   I   S   S   S   L   Y   I   E   *   I   L   N   H    539
ATG TAT TAC ATT TCA TGG TAC ATT AGT AGT TCT TTG TAT ATT GAA TAA ATA CTA AAT CAC  1617

L                                                                                540
CTA                                                                             1620
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FIG. 18B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/05606

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :536/23.1; 530/350, 387.1; 436/6, 69.1; 325, 320.1; 514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 530/350, 387.1; 436/6, 69.1; 325, 320.1; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database GENBANK, Accession Number D63815, SHIMIZU, A. et al. Human mRNA for rod photoreceptor protein, complete cds. Submitted 08 August 1995, see this nucleic acid sequence in relationship to SEQ ID NO:2 (amino acid residues 30-495), SEQ ID NO:5, nucleotides 37-1485), SEQ ID NO:51 (amino acid residues 1-446), and SEQ ID NO 64 (amino acid residues 1-466).	1-7, 9-14, 16-42

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 JULY 1999	19 AUG 1999

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	D. Lawrence for Karen Cochrane Carlson, Ph.D.
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/05606

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07H 17/00; C07K 14/00; A01N 43/04; A61K 35/14; C12Q 1/68; C12P 21/06; C12N 15/00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/05606

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 8, 15
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.